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SECTION OF GEOLOGICAL SCIENCES

IRONSHORE IN SOME WEST INDIAN ISLANDS*

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In tropical waters, where bedrock along the shore is Pleistocene reef material, there commonly develops a shore form characterized by dominant solution, rather than abrasion of the reef limestones. This zone of solution extends from a little below mean tide level inland as far as storms throw substantial amounts of sea water during wave break.

Residents of the West Indies often refer to this type of coast as "ironshore," a term suggested by its physical rather than its chemical attributes. Although the term is a local one, the feature it describes is widespread, and I have examined it in the Bahamas, the Cayman Islands, Cuba, and at a few points in south Florida.

As waves splash upon the old reef limestones, much of the water falls on slopes leading back to the sea. In its return the water may concentrate in chance irregularities on the rock surface, dissolving rill channels up to a foot in depth, vertical-sided or even undercut where layers of more rapidly soluble rock are encountered. The water falling in closed depressions on the surface enlarges the depressions by solution, the dissolved material being removed either in the flushing from a larger wave or, perhaps, by wind in the case of total evaporation. The resulting solution pits are therefore not like those of karst topography, since they lack underground drainage, but are comparable to the *tinajitas*, or etched potholes, found in the limestone of desert regions. As in *tinajitas*, the floor of the pit is nearly flat and the sides steep. Depths of the pits range up up to about four feet, with a like diameter for a single pit, although larger depressions are formed by the coalescence of two or more pits.

During times when the rock surface dries there is some redeposition of carbonates within the porosity of the limestone close to the surface. Thus there is formed, by chemical and perhaps also by biochemical action, a case-hardened surface that imparts surprising strength to the sharp ridges between pits, indurating them sufficiently to produce a metallic clink when struck. Anyone walking across such a surface soon realizes that the term ironshore is an apt one, indeed.

Thickness of the case-hardened layer varies from zero near mean tide level to about one half of an inch. The surface is usually a somber gray in color, and the indurated zone appears on a broken face as only a slightly darker shade of the light-colored reef rock.

*This paper, illustrated with slides, was presented at a meeting of the Section on May 4, 1959.

Examination of areas where the limestone surface is stripped clean for some distance inland demonstrates that solution is being carried on rapidly in the belt washed by sea water, but is less effective in the part washed principally by rain. Solution is most rapid on surfaces exposed to vigorous wave action, and is negligible to absent on the same type of rock in sheltered lagoons and bays. The level of most rapid solution is slightly above mean tide, and a pronounced notch may be produced at that height.

A most interesting area for the examination of ironshore may be seen along the west shore of Grand Cayman Island, south of Georgetown. Here the Pleistocene reef limestone is cut by two sets of vertical master joints placed nearly at right angles to each other. These master joints are spaced approximately 100 feet apart. Each system also has lesser joints, sometimes filled with secondary calcite, at 20- to 30-foot intervals. The joint pattern determines the general outline of the shore. Where weaker blocks have been eroded, re-entrants in the shore line provide landing places (locally known as *barcaderes*) for small boats.

The joint-determined sea cliff of old-reef limestone has a maximum height of 25 feet, of which as much as 10 feet may rise above mean tide level. The solution notch in its face is commonly 2 to 2.5 feet high, is centered about 4 inches above mean tide level for February, and is cut into the cliff to depths ranging from a few inches to 10 feet.

At the foot of the cliff, the bottom is a flat limestone surface with a few living corals and sparse patches of coarse limesand and pebbles.

In general appearance this shore line is indistinguishable from Pacific areas recently illustrated by Revelle and Emery (1957, pls. 225 to 227).

In the solution notch the rock surface is free from macroscopic organic encrustation, although attached algae are conspicuous both above and below. Gastropods such as *Tectarius* and *Littorina* are transient occupants of the notch zone, and chiton populations as high as six per square yard were common. Some scrapings from the apparently barren rock surface of the notch contained minute amounts of organic material, presumably algal; other scrapings were entirely of mineral material.

Rate of Solution

In two cases, where insoluble materials have been placed against cut surfaces in the solution notch at a known date, it was possible to obtain rough figures for the rate of limestone solution. On the west end of Grand Bahama Island the surface was being removed at a rate of about one tenth of an inch per year, and on the southwest shore of Grand Cayman the rate was about one quarter of an inch per year. Since other nearby areas appeared to be undergoing still more rapid solution, these figures are not likely to represent maximum rates.

Method of Solution

The descriptions of tropical sea waters found in most reference works stress the fact that such waters are saturated with respect to carbonates, and therefore not likely to dissolve limestone. The ironshore, with its water level notch, obviously must involve some special situation. Revelle and Emery (1957) have discussed this at length, following studies at Bikini and elsewhere in the Pacific. Their tentatively suggested explanations are:

(1) Diurnal changes in water composition at the rock face, depending in part on varying rates of photosynthesis or respiration in the attached life. This seems unsatisfactory, since solution proceeds fastest where there is least attached organic matter.

(2) Simultaneous reactions, one involving precipitation of carbonate, and the other and more rapid reaction producing solution. This still remains unproved.

Wiens (1959) has summarized the opinions offered by other recent writers on the problem, again without reaching a convincing explanation.

I have emulated other investigators in making many determinations of *p*H in the waters studied, using Hydrion papers. These tests were made in December, February, and July. Aside from special situations, such as occur in small basins, the indicated *p*H varied between 7.3 and 7.7, a range hardly consonant with rapid carbonate solution. Only 2 readings below 7.3 were recorded, both early in the morning, and both after vigorous storms. These isolated readings of 7.2 were taken at spots where the bottom falls rapidly offshore to a considerable depth. Since all other readings at these two spots were of the order of 7.5, there is some indication that the storms may have moved away the usual surface water, with consequent upwelling from depth. A systematic program of measurements at places such as these might reveal sporadic upwellings, such as those recently reported for the east Florida coast by Taylor and Stewart (1959).

It should be emphasized that solution occurs, not only in the notch, but all along the shore. Wherever the ironshore is best developed there is a remarkable absence of fine limestone particles, either as precipitated material or comminuted debris. In the absence of other mineral materials, amazing clarity of water results. On Grand Cayman, for example, off the ironshore coast the bottom can be seen from the air at depths indicated on charts to be as great as 150 feet. This clarity of water suggests that the finest limestone debris dissolves as fast as it is broken from the thin edges of the ironshore.

None of the ideas discussed above explains the undebatable fact that solution is more rapid at approximately mean sea level than elsewhere. I feel that the answer is to be found neither in *p*H determinations, nor in calculation of carbonate solubility products, as previously attempted. What we should investigate is the pCO_2 (pressure of dissolved CO_2) of

the water in the area of wave break against the limestone. Does the agitated water of this zone acquire additional CO_2 from the atmosphere during wave break? Does such CO_2, aided in its solution by mechanical entrapment, furnish a small but constant additional supply that produces rapid solution of the limestone? Answers to these questions may solve the problem. For an example of a possibly parallel situation, see Loomis' recent (1959) article "The Sex Gas of Hydra," in which an interpretation was untenable on the basis of *p*H data, but reasonable when pCO_2 was considered instead. A similar approach, concerned with solubility of limestone by fresh water, was explored by Adams and Swinnerton in 1937.

In the course of any investigation some tools will be found useful, others useless. This particular problem seems to be one in which too many investigators have relied too heavily on *p*H measurements to no avail.

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THE NEW YORK ACADEMY OF SCIENCES

SECTION OF CHEMICAL SCIENCES

HELICAL ARRANGEMENTS IN SYNTHETIC AND NATURAL POLYNUCLEOTIDES*

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The last decade has witnessed the accumulation of a wealth of evidence that suggests that the control of specificity in biological systems is mediated through information contained in the chemical structure of the group of biomacromolecules known as nucleic acids. The role of the transmission of the character of the species from generation to generation has been relegated by Nature to the desoxyribonucleic acids (DNA), while the ribonucleic acids (RNA) appear to have been made responsible for the unfolding of this information within a generation. It is reasonable to assume that information is coded within these polymers by way of the sequence of their monomer components, the nucleotides; there is, nevertheless, convincing evidence that the biological activities of these macromolecules are dependent also on the uniqueness of their secondary and tertiary structure. If we are to gain further insight into the molecular basis of heredity, the duplication of genes, and the synthesis of specific macromolecules of cells, such as proteins, it seems crucial to extend our understanding of the configurational properties of the nucleic acids.

Nucleic acids are linear polyelectrolytes, polymers of nucleotides, linked through diester phosphate bonds between the 3' and 5' carbons of the pentoses of adjacent nucleotides to form a sugar-phosphate backbone. In DNA the sugar is 2-deoxyribose, and in RNA it is ribose. The nucleic acids have three bases in common: adenine, guanine, and cytosine. RNA contains uracil as well, and DNA thymine instead; occasionally, other bases occur as minor constituents.

Our knowledge of the macromolecular configuration of DNA is at present on firm footing, having yielded to the combined efforts of X-ray crystallographic analysis,[1] hydrodynamic studies, and other physicochemical methods.[2,3] The essential features have been embodied in the now familiar Crick-Watson model.[4] In this structure there exist two interwinding polynucleotide chains, held together by hydrogen bonds through the medium of specific base pairs, to give an over-all helical configuration, with the bases on the inside and the phosphate groups on the periphery.

In contrast to DNA, both crystallographic[5] and hydrodynamic efforts,

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until very recently, had failed to provide us with any reasonable picture of the macromolecular structure of RNA. During the present year, however, the correlation of findings from several different investigations has enabled us to formulate, for the first time, some concepts of the three-dimensional conformation of RNA.[6-11]

The direct investigation of the physicochemical properties of natural RNAs has afforded us only part of the information on which these views are based. Much of our insight into polyribonucleotide structure has been derived from a systematic study of the properties and interactions of synthetic homo- and copolymers of ribonucleotides. This approach first became possible about four years ago when Grunberg-Manago and Ochoa[12] discovered an enzyme that catalyzes the polymerization of ribonucleoside diphosphates to polyribonucleotides. Since the enzyme shows no specificity for the bases, the reaction can be used to synthesize polymers and copolymers of the bases normally found in RNA according to design. When it became apparent from the studies of Heppel *et al.*[13] that the sugar-phosphate backbone of these model polymers is the same chemically as that for RNA, Paul Doty and I commenced, in 1956, a study of their configurational properties in the hope that it might provide an oblique approach to the solution of the macromolecular structure of RNA.

The availability of the single polymers and copolymers of known composition would enable us, presumably, to ascertain the types of interactions possible as a result of the active groups, particularly those of the purine and pyrimidine rings. Our aim was to discover the various possible configurations that can be assumed by these polynucleotides, pure and mixed, and the conditions that permit the stabilization of the more unique crystalline configurations. This is of interest even when the conditions found are far removed from those of the physiological state, since we must consider the possibility that such configurations could be stabilized by interaction with other cell constituents.

In keeping with our purpose, we attempted to define the different molecular configurations that can be assumed by polyriboadenylic acid, poly A, polyuridylic acid, poly U, and polycytidylic acid, poly C, in aqueous solution at different *p*H and ionic strength conditions. This led to the discovery that poly A[10,11] and poly C,[14] but not poly U,[15] can undergo reversibly a helix-coil transition. From these and other studies[7,8,16,17] it became evident that interactions between and within polynucleotide chains result primarily from the formation of hydrogen bonds between pairs of adjacent or neighboring purines and pyrimidines, and that such interactions can involve many more pairs of bases than those prescribed by Watson and Crick for DNA. Indeed, this possibility of more than twenty such interactions has been shown by model building.[18]

An important development stemming from the physicochemical studies of the synthetic polynucleotides has been our recent finding that macromolecular configuration makes a significant contribution to the optical

rotatory properties of polynucleotides.[7,8,11,19] In what follows I propose to consider briefly these observations in the light of other information on polynucleotide structure and to present our reasons for interpreting the optical rotatory properties of both DNA and RNA as evidence of helical structure.

It is current information that the alkaline or enzymatic hydrolysis of nucleic acids to their constituent nucleotides is accompanied by a large increase in ultraviolet absorption and that a major part of this hyperchromic effect can be induced merely be elevating the temperature to near 100° (and recording the absorption at the elevated temperature). Moreover, since, in the case of DNA this hyperchromic change is followed by an irreversible collapse of the organized helical structure, the view has emerged that the magnitude of the effect is an indication of the degree of hydrogen bonding between bases within the polynucleotide. We have explored the profile and the magnitude of this hyperchromic change induced on heating, and have used this information to classify synthetic and natural polynucleotides into three classes. Our confidence in the validity of the classification stems from elaborate physicochemical studies, a description of which is not appropriate to this discussion.

Thus far, only a single example of the first class has been found: poly U. This polymer can be described as a randomly coiled, nonhydrogen-bonded single chain and it shows only an insignificant (2 per cent) hyperchromic effect on being heated. In the second group are included the irregularly coiled but partially hydrogen-bonded single chains. Examples include all natural RNAs examined, and poly A and poly C at neutral *p*H. Upon examining the optical density-temperature profile of poly A at neutral *p*H,[11] it is observed that the hyperchromic change measured at the elevated temperature is gradual, extending over some 60°. In addition, the change is smaller in magnitude than that observed for the two-stranded helical form of poly A, the latter being an example of the third class: the two- and three-stranded completely helical polynucleotides. Native DNA also falls into this class. In these cases, the helices collapse over a very narrow temperature range (less than 10°) and the hyperchromic change is greater than that for the coil forms of class II, indicative of the higher content of hydrogen-bonded bases.

From these observations it should be apparent that polynucleotides in class II have a high proportion of their bases in some types of hydrogen-bonded linkages that are thermolabile and, incidentally, also labile to the competitive effect of urea. In view of this, it is germane to consider whether the hydrogen-bonded bases in this class of polynucleotides are randomly distributed within the single chains or are, instead, part of some organized structural elements, such as helical regions, in these single chains. Aware of the success with which the methods of optical rotation and rotatory dispersion have been used to detect and quantitate the pres-

ence of helical regions in synthetic polypeptides and proteins,[3,20] we thus investigated the polynucleotides.

Optical rotatory measurements of polynucleotides in solution were made at the sodium D line at room and at high temperatures and, in some cases, the change in rotation was followed as the temperature was elevated. Some of these data are recorded in TABLE 1. In considering the data, it is well to keep in mind the fact that the specific rotation, $[\alpha]_D$, of the mononucleotides clusters around 0^0. It is seen that poly U (of class I) also has a

TABLE 1

OPTICAL ROTATION OF POLYRIBONUCLEOTIDES

Polymer	Temperature	$[\alpha]_D$
U coil	22°	−8°
A coil	22°	155°
	90°	−5°
+ 8M urea	22°	75°
A helix	22°	300°
(*A* + *U*) helix	22°	300°
	70°	23°

value in this vicinity. At the other extreme (class III) poly A helix and poly (A + U) helix have very large positive rotations. This is particularly striking in the case of poly (A + U), since it is made of one strand having an initial rotation of 155° and another of -8°. Obviously, when the poly U chain is transformed into a helical conformation, it contributes with as high a positive rotation as the poly A chain. This is clearly a unique configurational effect, completely separate from that due to asymmetrical centers in the optically active ribose. Furthermore, when the poly (A + U) helix is denatured at high temperature, the rotation drops to near 0^0, as does also the poly A coil (of class II), which has an intermediate initial rotation at room temperature. Another important point not apparent from the table is that, over the course of the thermally induced changes, the decrease in positive rotation, in all cases studied, parallels the increase in ultraviolet absorption. From this series of observations it should be evident that hydrogen-bond content is associated with high positive rotation.

Similar findings have been made with natural polynucleotides. Boedtker has observed that the $[\alpha]_D$ of RNA from tobacco mosaic virus at room temperature is just slightly larger than that of the poly A coil, and that it also approaches 0^0 as the hydrogen bonds are broken at high temperature.

For DNA, the change in $[\alpha]_D$ on complete denaturation at high temperature is only about 100^0 (126^0 to 26^0) instead of the nearly 300^0 decrease seen with the completely helical polyribonucleotides. This might be due to differences in the structure and orientation of the two pentoses with respect to the helix axis.

From this series of observations it may be apparent that high hydrogen-bond content is only incidentally associated with large positive rotation. The magnitude of the rotational change accompanying the thermal disruption of the hydrogen bonds is so large that the origin of the high positive rotation must lie in some type of regular or ordered repeating three-dimensional arrangement of atoms that provide for oscillation of polarized light. It is easy to recognize such a pattern in the class III polynucleotides, since they are known to be complete helices. In the class II polynucleotides, where 40 to 60 per cent of the bases are hydrogen-bonded, the positive rotation is similarly proportional to the rotation of the completely helical molecules. On this basis, the positive rotation of the partially hydrogen-bonded coil polynucleotides is ascribed to the presence of short helical regions held together by hydrogen bonds between the bases.

As another means of detecting helical regions, we have begun to explore the rotatory dispersion, that is, the wave-length dependence of optical rotation of polynucleotides. An earlier report[21] claimed that the dispersion is normal for native DNA. With R. Gorn, we have found that the dispersion follows a one-term Drude function in the region from 360 to 630 $m\mu$, but above and below this region there are deviations. Of particular interest is the finding that the rotation is anomalous in the region of absorption of the purines and pyrimidines, becoming at first highly positive and then changing sign in a manner defined by the Cotten effect.[22] Since the purines and pyrimidines contain no asymmetric atoms and are therefore not themselves optically active, they must contribute to the optical activity of DNA in a manner that definitely reflects their behavior as oscillators in a helical arrangement. Since the unoriented benzene groups do not behave in this fashion in the α helix of polybenzylglutamate,[23] our interpretation would seem to rest on firm grounds.

From the optical rotatory studies described the argument emerges that there are short helical regions in the single strands of isolated RNA in solution. We are not yet in a position to detail the structure of these helical regions. Perhaps they originate from short hairpin loops within the single chain, stabilized by hydrogen bonds made possible by particular sequences of sterically appropriate purines and pyrimidines. I do not wish to imply that we can now evaluate the biological significance of these helical arrangements. Perhaps our findings are only a suggestion of the three-dimensional arrangements that Nature calls on RNA to assume. I prefer to imagine that the helical arrangements that have been discovered bear some closer relation to the kinds of conformations that these molecules assume in some of their biological roles.

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SECTION OF BIOLOGICAL AND MEDICAL SCIENCES

CONDITIONING FACTORS FOR CARDIAC NECROSES*

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It has long been known that biological reactions depend, not only upon the nature of the evocative stimulus, but also upon other factors (for example, heredity, age, and diet), which condition reactivity. Such conditioning actions play a particularly important role, not only during the General-Adaptation-Syndrome, but also in the pathogenesis of various diseases. More particularly, it was demonstrated that hormones can play a decisive role in the development of several nonendocrine diseases, that is, of maladies not primarily due to derangements originating in the endocrine glands themselves.[1]

Consequently, in the course of our recent studies concerning necrotizing cardiopathies the question arose whether there are conditioning factors capable of selectively sensitizing or desensitizing the heart to the effect of various agents known to be potentially cardiotoxic. Let us merely point out here that we employed as pathogens: (1) noradrenalin and vasopressin as agents capable of producing "spotty myolysis" with disappearance of muscle-fiber segments, without extensive necroses or inflammation; (2) stressor agents, such as forced restraint, quadriplegia, hot and cold baths, or surgical trauma, which likewise produce, but only occasionally, small necrotic foci (for example, "micronecroses"); (3) papain, because this proteolytic enzyme preparation elicits acute, miliary, disseminated myocardial necroses; (4) plasmocid (a rather toxic and now obsolete antimalarial), since it produces large, patchy myocardial necroses and myocarditic lesions; (5) dihydrotachysterol (DHT), a steroid of the vitamin D group, because it causes intense calcium deposition in the heart and in the coronary arteries; and (6) 2_{α}-methyl-9_{α}-chlorocortisol (Me-Cl-COL) + NaH_2PO_4, since this combined treatment is highly effective in producing a typical electrolyte-steroid-cardiopathy (ESCN), characterized by massive, infarctlike cardiac necroses with secondary inflammation, but without any demonstrable histological change in the structure of the coronary arteries. The histological characteristics of these experimental cardiopathies, which more or less closely

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The Section of Physical Sciences held a meeting on May 12, 1959, at which Sidney Borowitz of the Department of Physics, New York University, New York, N. Y., presented a paper on "Atomic Processes in the Upper Atmosphere." This paper will not be published by the Academy.

imitate certain spontaneous myocardial changes of man, have been described in detail elsewhere.[2] Furthermore, in addition to those enumerated above, several other agents were also tried. Some (for example, Na-fluoroacetate, pentamethylentetrazol, and arsphenamine) were used particularly because of contradictory reports in the literature concerning their ability to produce cardiac necroses; others (for example, diisopropylfluorophosphate), because they were not known to produce this type of cardiac damage. We felt that comparative studies with such a wide variety of potentially cardiotoxic agents would give us some information concerning common features in the pathogenesis not only of cardiac necroses in general, but especially of those spontaneous diseases of man that are not accompanied by acute occlusion of the coronary vessels.

Positive Conditioning Actions (Sensitization)

Among the sensitizing agents, *corticoids* (that is, desoxycorticosterone, hydrocortisone, and halocorticoids) appear to be particularly active conditioning factors in the production of cardiac necroses with certain Na-salts (especially NaH_2PO_4, Na_2SO_4 and $NaClO_4$). The administration of a combination of both mineralo- and glucocorticoid substances, or of a single compound possessing both these activities (for example, Me-Cl-COL) is extremely effective in producing the ESCN, as well as in sensitizing the heart to a great variety of essentially different stressor agents.[3] For example, after pretreatment with Me-Cl-COL, pentamethylentetrazol, Na-fluoroacetate, and diisopropylfluorophosphate all consistently elicited cardiac necroses in the rat, although neither these compounds nor the steroid showed any cardiotoxic properties when given alone. We note that, among the halogenated corticoids tested, only triamcinolone (a pure glucocorticoid) failed to condition the cardiac muscle to the toxic effect of Na salts. On the other hand, however, this hormone was found to be especially active in the production of "spotty myolysis," when given simultaneously with noradrenalin to intact or adrenalectomized animals.[4]

Experiments dealing with the participation of electrolytes in the production of cardiopathies give a number of interesting results. Like Me-Cl-COL, Na_2HPO_4 greatly predisposes the cardiac muscle to the necrotizing effects of proteolytic enzymes (papain, ficin), as well as to that of arsphenamine, while the cardiotoxic actions of other agents (noradrenalin, vasopressin) were not significantly influenced by this electrolyte.[5,6] Only certain Na salts (for example, phosphates, sulfates, sulfites, and perchlorate) were effective in inducing an ESCN; therefore, we concluded that both the anion and the cation are important factors in the pathogenesis of this type of experimental cardiac disease. It was also noted that the same steroid can predispose the rat for the production of qualitatively dissimilar cardiac lesions, but different electrolytes are necessary to elicit one or another type of change. For example, admin-

istration of NaH_2PO_4 or Ca-acetate can alter the calcifying and slight necrotizing actions of DHT, so that a suppurative, acute myocarditis results. While the concurrent administration of desoxycorticosterone + Na_2HPO_4 produces large patches of infarctlike necroses (ESCN) in the rat, the same steroid when given with NaCl elicits quite a different type of lesion: instead of necroses, a typical hyalinizing myocarditis occurs.[2] We were especially impressed by the observation that NaCl – which usually exerts a definite, though mild, desensitizing effect upon the production of an ESCN – greatly augments the sensitivity of the heart to the production of necroses by *stressor agents*, such as forced restraint.[7] In order to study further the possible key role played by Na in the biochemical derangements of stress-induced infarctoid cardiopathies we performed the following experiments.

Three hundred and sixty female Sprague-Dawley rats, with a mean initial body weight of 98 g. (range: 92 – 110 g.), were subdivided into 36 equal groups and treated as indicated in TABLE 1. Me-Cl-COL was administered subcutaneously in the form of its acetate, as a microcrystal suspension of 100 μg. in 0.2 ml. of water, once daily. All the Na salts were given by stomach tube, at the dose of 2mM in 2 ml. of water, twice daily. Neuromuscular stress was induced by restraining the rats on a board with adhesive tape, for a period of 17 hours, on the fifth day of the experiment. The animals of all groups were killed with chloroform on the sixth day. The hearts were fixed in neutral formalin and embedded in paraffin for subsequent staining with our fuchsin technique.[2] The intensity of the necroses was assessed in terms of an arbitrary scale of 0 to 3, and the means of these readings (with standard errors) as well as the incidence of the lesions are listed in TABLE 1.

It may be seen from the figures given in TABLE 1 that the various Na salts tested are well tolerated even by rats simultaneously treated with Me-Cl-COL or a neuromuscular stressor. However, these same salts produce massive and sometimes fatal myocardial necroses in rats exposed to stress after conditioning with Me-Cl-COL. It appears that during stress the metabolism of certain otherwise innocuous Na salts and/or steroids is so altered that these substances acquire severe cardiotoxic properties. Other investigations revealed, furthermore, that the precipitating effect of neuromuscular effort is not specific, since a variety of other stressors (hot or cold baths, surgical trauma, adrenaline) also produce myocardial necroses after electrolyte-steroid conditioning. The cardiotoxic effects of other agents (for example, papain, plasmocid, or arsphenamine) were also significantly enhanced by simultaneous exposure to stress. All these experiments demonstrated that, under certain conditions, stress, corticoids, and a number of Na salts exert a necrotizing effect on the heart muscle and also influence the cardiotoxic effects of many otherwise unrelated agents.

TABLE 1

THE ROLE OF NA SALTS IN STRESS-INDUCED CARDIAC NECROSES

Group	Treatment	Cardiac necroses	
		Grade (0 to 3)	Incidence (%)
1	None	0	0
2	$NaHCO_3$	0	0
3	Na-butyrate	0	0
4	Na-glutamate	0	0
5	Na-glycerophosphate	0	0
6	Na-formate	0	0
7	Na-pyruvate	0	0
8	Na-succinate	0	0
9	Na-tartrate	0	0
10	Me-Cl-COL	0	0
11	Me-Cl-COL + $NaHCO_3$	0.1 ± 0.10	10
12	Me-Cl-COL + Na-butyrate	0.1 ± 0.10	10
13	Me-Cl-COL + Na-glutamate	0	
14	Me-Cl-COL + Na-glycerophosphate	0.2 ± 0.20	20
15	Me-Cl-COL + Na-formate	0	
16	Me-Cl-COL + Na-pyruvate	0	
17	Me-Cl-COL + Na-succinate	0.3 ± 0.16	20
18	Me-Cl-COL + Na-tartrate	0.1 ± 0.10	10
19	Restraint	0	
20	Restraint + $NaHCO_3$	0.2 ± 0.20	10
21	Restraint + Na-butyrate	0.3 ± 0.15	30
22	Restraint + Na-glutamate	0	
23	Restraint + Na-glycerophosphate	0.2 ± 0.18	20
24	Restraint + Na-formate	0.4 ± 0.16	30
25	Restraint + Na-pyruvate	0.7 ± 0.28	50
26	Restraint + Na-succinate	0.4 ± 0.15	40
27	Restraint + Na-tartrate	0.3 ± 0.17	30
28	Restraint + Me-Cl-COL	0.2 ± 0.25	20
29	Restraint + Me-Cl-COL + $NaHCO_3$	2.4 ± 0.20	100
30	Restraint + Me-Cl-COL + Na-butyrate	2.9 ± 0.10	100
31	Restraint + Me-Cl-COL + Na-glutamate	2.7 ± 0.25	100
32	Restraint + Me-Cl-COL + Na-glycerophosphate	2.6 ± 0.32	100
33	Restraint + Me-Cl-COL + Na-formate	3.0 ± 0	100
34	Restraint + Me-Cl-COL + Na-pyruvate	2.5 ± 0.22	100
35	Restraint + Me-Cl-COL + Na-succinate	2.7 ± 0.18	100
36	Restraint + Me-Cl-COL + Na-tartrate	3.0 ± 0	100

Observations on animals subjected to dietary deficiencies showed that the same "sensitizing Na salts" (Na_2HPO_4, Na_2SO_4, $NaClO_4$) that produce an ESCN when administered with certain corticoids exert similar necrotizing effects in rats sensitized by a K-deficient diet.[8] In both these conditions, the anion must play a decisive role, since NaCl in amounts equivalent to those Na salts mentioned is ineffective. Subsequent investigations revealed the fact that in rats maintained for only one week on a K-deficient diet (which at the same time contained only minimum maintenance levels of Mg) the myocardium showed no detectable structural alterations. However, in such animals massive myocardial necroses could be produced by otherwise ineffective doses of noradrenalin, vasopressin, thyroxine, DHT, plasmocid, forced restraint, cold or hot baths, vagotomy, quadriplegia, and intestinal trauma.[9] These results suggested that a brief period of nutritional K deficiency can selectively condition the myocardium for the cardiotoxic effect of stress. On the other hand, increased intake of fat or carbohydrates, as well as natural foods rich in these dietary constituents, very effectively enhances the incidence and severity of the ESCN and also of the acute myocarditis that results from administration of DHT + Ca-acetate.[2,10]

All the experiments summarized here were performed on healthy young animals with normal cardiac vessels, while spontaneous infarcts tend to occur with the greatest frequency in middle-aged or older men, in whom a certain degree of coronary sclerosis may have already developed. In order to produce similar experimental conditions, rats were pretreated with DHT, and sclerosis and hardening of the coronary system (Mönckeberg type) was thus induced. It was found that in these artificially "aged" animals, otherwise ineffective doses of noradrenalin, vasopressin, papain, cold baths, or Me-Cl-COL + NaH_2PO_4 all produced particularly extensive, macroscopically visible patches of necrosis throughout the heart. Quite unexpectedly, it was also noted after sensitization with DHT that intense proliferative and eventually obstructive changes develop in all parts of the coronary tree during the action of the cardiotoxic agents enumerated above.[2,11] The mechanism of this vascular conditioning is not yet clear, but the observations suggest that the toxic effect of various agents, especially that of certain electrolyte-steroid interactions, is not necessarily limited to the cardiac muscle itself, but may also participate in the production of obstructive coronary lesions.

Subsequent studies were then performed to examine the importance of the *age factor* in the production of various experimental cardiac lesions. It was revealed that papain, noradrenalin, vasopressin, DHT, and combined treatment with Me-Cl-COL + Na_2HPO_4 induce much more pronounced cardiac lesions in old than in young rats when the dose of these substances is adjusted to body weight. On the other hand, stressors, such as restraint and quadriplegia, as well as plasmocid, which is also

singularly resistant to the various corticoids and Na salts, were about equally effective in young and in old animals.[12] Still, in view of the enormous importance of cardiovascular disease in gerontology, it is of interest that a variety of experimental cardiac lesions depends so largely upon the age factor.

Negative Conditioning Actions (Desensitization)

Perhaps one of the most striking results of our investigations was the demonstration that the production of necrotizing cardiopathies by so many agents is almost uniformly prevented by certain factors through negative conditioning. As the role of high blood pressure in the pathogenesis of hyalinizing myocarditis had previously been well established,[2] we were interested to see the effect of hemodynamic changes in other cardiopathies. Various histological types of cardiac lesions (necrosis, inflammation, fuchsinophilic degeneration, calcification) have been produced in rats by papain, plasmocid, the stress of ice-cold baths, simultaneous treatment with DHT + NaH_2PO_4, or by combined administration of Me-Cl-COL + Na_2HPO_4. Irrespective of the morphologic appearance of the lesions thus induced, hemorrhage exerted a pronounced prophylactic effect against all these agents except plasmocid, while noradrenalin aggravated the changes thus induced by all agents, including plasmocid. It is worthy of mention that the severe lesions produced by noradrenalin given in combination with the various agents just cited do not exhibit the histological characteristics of noradrenalin overdosage ("spotty myolysis"), but always retain the specific histological features characteristic of the agent given in combination with the adrenergic hormone.[12,13] It was postulated that hemorrhage acts by decreasing, and noradrenalin by increasing, cardiac work. This view is further supported by the observation that in severe shock (produced by spinal-cord transection) the pulse may be weakened and the peripheral resistance lowered to such a degree that necrosis does not occur.[14]

Independent investigations on the influence of age as well as that of pregnancy upon the development of various necrotizing cardiopathies revealed that youth protects the heart against essentially the same cardiotoxic agents as does pregnancy. Thus, pregnant animals proved to be particularly resistant to noradrenalin, vasopressin, papain, and ESCN, but not to restraint, cold, or quadriplegia.[15]

From a clinical point of view, perhaps the most important finding is that the oral administration of various chlorides, and especially of KCl and $MgCl_2$, is very effective in desensitizing the cardiac muscle. KCl and $MgCl_2$ prevent the occurrence not only of the typical ESCN induced by corticoids and sensitizing Na salts, but also of the suppurative myocarditis that is normally elicited by combined treatment with DHT + NaH_2PO_4, the necrotizing myocarditis that develops in corticoid-

conditioned animals during exposure to various stressors, and even those cardiac necroses that follow (presumably as a result of a direct proteolytic action) intravenous treatment with a protease.[2,16]

In view of our findings concerning the interactions of anions and cations in the production of experimental cardiopathies, and particularly further to investigate the protective effect of chlorides, we wished to determine: (1) whether the degree of cardiac necroses elicited with certain Na salts (for example, $NaClO_4$) is independent of sensitization by K or Mg deficiency; and (2) whether the morbid changes so induced would be prevented equally with various chlorides.

One hundred and seventy female Sprague-Dawley rats, with a mean initial body weight of 45 gm. (range: 40 to 54 gm.) were subdivided into 17 equal groups and treated as indicated in TABLE 2. The animals of Groups 2 to 9 were maintained on a K-deficient diet, and those of Groups 10 to 17 on an Mg.-deficient diet, respectively, for 5 days prior to as well as during the entire experiment. The composition of these deficient diets* is described elsewhere.[17] The control rats, Group 1, were main-

TABLE 2

SENSITIZATION WITH K- OR MG-DEFICIENCY FOR THE NECROTIZING EFFECT OF $NaClO_4$: THE ROLE OF CHLORIDES IN ITS PREVENTION

Group	Treatment	Cardiac necroses	
		Grade (0 to 3)	Incidence (%)
1	$NaClO_4$	0	0
2	K-deficient diet	0	0
3	K-deficient diet + $NaClO_4$	2.8 ± 0.18	100
4	K-deficient diet + $NaClO_4$ + KCl	0	0
5	K-deficient diet + $NaClO_4$ + $MgCl_2$	0.4 ± 0.20	30
6	K-deficient diet + $NaClO_4$ + NaCl	2.1 ± 0.32	80
7	K-deficient diet + $NaClO_4$ + $CaCl_2$	0.6 ± 0.25	30
8	K-deficient diet + $NaClO_4$ + NH_4Cl	0.1 ± 0.10	10
9	K-deficient diet + $NaClO_4$ + $MgCl_2$ + NaCl + $CaCl_2$ + NH_4Cl	0.2 ± 0.15	10
10	Mg-deficient diet	0	0
11	Mg-deficient diet + $NaClO_4$	1.5 ± 0.27	80
12	Mg-deficient diet + $NaClO_4$ + KCl	0.3 ± 0.17	20
13	Mg-deficient diet + $NaClO_4$ + $MgCl_2$	0	0
14	Mg-deficient diet + $NaClO_4$ + NaCl	0.4 ± 0.18	40
15	Mg-deficient diet + $NaClO_4$ + $CaCl_2$	0.2 ± 0.15	10
16	Mg-deficient diet + $NaClO_4$ + NH_4Cl	0	0
17	Mg-deficient diet + $NaClO_4$ + KCl + NaCl + $CaCl_2$ + NH_4Cl	0	0

*Supplied by General Biochemicals, Inc., Chagrin Falls, Ohio.

tained on the normal laboratory food, Purina Fox Chow. All electrolytes were given in 2 ml. of water through a stomach tube twice daily. Each individual dose of $NaClO_4$ was 0.5 mM, while that of the chlorides or their mixtures consisted of an amount equal to the chloride content of 0.25 mM KCl. The experiment was terminated on the third day (Groups 2 to 9) or on the seventh day (Groups 1, 10 to 17) and the cardiac changes were evaluated as described earlier in the text.

As the data in TABLE 2 show, after sensitization with either a K-deficient or Mg-deficient diet a short period of treatment with $NaClO_4$ sufficed to cause widespread cardiac necroses in the vast majority of the experimental animals. On the other hand, this dietary conditioning to the cardiotoxic effect of $NaClO_4$ was abolished or at least markedly diminished by concurrent administration of various chlorides. Only the prophylactic effect of NaCl was not significant in the K-deficient animals. It is particularly interesting to note that the sensitizing action of K deficiency was effectively decreased by, among others, $MgCl_2$, and vice versa. Consequently, the anion must play an important role in this preventative reaction, since K and Mg ions are believed to be in many respects even antagonistic. This hypothesis is supported by many of our earlier studies, which showed that none of the salts other than chlorides is highly effective in the prevention of an ESCN; in this respect, KCl and $MgCl_2$ are equally potent.[2]

Summary

The primary object of this paper is to summarize our observations on the experimental cardiopathies that depend largely upon conditioning factors, which sensitize (corticoids, Na salts, stress, dietary deficiencies, coronary sclerosis, age) or desensitize (hemodynamic changes, pregnancy, chlorides) the heart to the production of cardiac necroses by the most diverse agents. Additional recent experiments, concerning the interactions of corticoids, electrolytes, and stress gave the following results:

(1) During stress, the metabolism of certain otherwise innocous Na salts and/or steroids is so altered that these substances acquire severe cardiotoxic properties.

(2) K or Mg deficiency equally sensitizes the heart to the cardiotoxic effect of $NaClO_4$.

(3) The sensitizing effects of both these dietary deficiencies are significantly diminished by various chlorides; among these, KCl is as active in Mg-deficient animals as is $MgCl_2$ in rats kept on a diet low in K.

The results of all these investigations suggest that there may be some common pathway in the mechanism through which various agents influence the heart muscle, thereby producing necroses and/or inflammation.

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DIVISION OF NUCLEAR PHYSICS

THE CHEMISTRY OF FISSION PRODUCTS IN A MOLTEN METAL NUCLEAR FUEL*

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Introduction

A number of different molten metal systems have been proposed as fuels for nuclear reactors. These include both homogeneous liquids and dispersions of solids in liquid metals. Possible solution fuels are the systems U-Bi, Pu-Bi, U-Al, Pu-Fe; for dispersions, there have been considered UO_2 in Bi and in Na, and uranium carbides in the same media. Of all these, by far the most work has been done on U-Bi. This system has nuclear properties that make it suitable for use in a thermal breeding cycle, in which U^{233} and Th^{232} are the fissile and fertile species; and since it is a solution, certain technical problems associated with dispersions are avoided. It has a conveniently low melting point; pure Bi melts at 271° C., and the addition of small quantities of U raises the melting point very little. Only small quantities are required; in a suitably designed reactor, a self-sustaining chain reaction will occur in a Bi solution containing less than 1000 ppm of U. The solubility of U in Bi at 500° C., a convenient operating temperature, is about 6500 ppm.

Various reactor designs have been proposed that utilize a U-Bi solution as a fuel. In some, the liquid alloy serves a dual purpose, acting both as fuel and heat-transport medium; that is, it circulates continuously between the reactor proper and a heat exchanger. In others it remains within the reactor where it is cooled by the circulation of a secondary coolant, which can be either a gas or a liquid metal, the heat being transferred through a suitable wall interposed between fuel and coolant. Even in the latter case the fuel would be connected to apparatus outside the reactor in such a way that it could be replaced or, sometimes, subjected to certain treatments in place without shutting down the reactor.

The composition of the fuel will be governed by the type of reactor for which it is intended, and the nature of the surfaces with which it must be compatible. For example, if a fuel is to circulate through a steel heat exchanger, with a 75° C. temperature differential, severe corrosion will occur unless small quantities of Mg and Zr are added to the liquid; they act as very effective inhibitors. The amount of U in the solution will be governed by reactor physics considerations and by the solubility of U at

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the coldest point in the system. It so happens that most of the work on the chemistry of liquid Bi fuels has been done in connection with a reactor design that calls for a solution of the following composition:

Uranium	600 to 1000 ppm
Zirconium	100 to 300 ppm
Magnesium	50 to 1000 ppm
Bismuth	rest

It was therefore this alloy that was used in most of the experiments on fission-product removal.

Methods for removing fission products were sought very early in the work on liquid metal fuels. In fact, the possibility of continuously removing fission products – an operation impossible in the case of solid fuels – was one of the chief stimuli in the early development of this type of fuel. The value of eta for U^{233} is only 2.27, so that neutron losses must be held to a minimum if one-for-one breeding is to be attained on the thorium cycle. Prompt removal of neutron-absorbing fission products would help in achieving this goal. There are other reasons for removing fission products, such as reducing the radioactive hazard in case of a reactor spill and the fact that some fission products affect the solubility of fuel constituents, but the overriding reason is neutron economy. TABLE 1 gives the yields and cross sections of the most important nuclides. In a high-flux reactor most of the Xe^{135} atoms will capture a neutron before they decay, thus causing a neutron loss approaching 7 per cent. Immediate removal is necessary; consequently, the behavior of xenon in liquid metal fuels has been the subject of special study, as described below. The other species in the table can be allowed to remain in the reactor somewhat longer, but not indefinitely; their chemistry has also been investigated.

Volatile Fission Products

The lowest Xe concentration that it may be feasible to maintain in a liquid metal fuel reactor (LMFR) core depends on the solubility of Xe in the liquid metal fuel and on the extent to which Xe is adsorbed on and absorbed by the graphite constituting the reactor core.

The solubility of Xe in a fuel of a composition in the range given in the introduction is essentially its solubility in pure Bi. This has been measured by Mitra and Bonilla,[1] who have reported an average value of 3.7×10^{-7} atom fraction at 1 atm. and 500° C. Hewitt *et al.*[2] have obtained solubilities in the range of 1.356×10^{-9} to 97.6×10^{-9} atomic fraction at 500° C. by mass spectrograph analysis and 0.08×10^{-10} to 0.51×10^{-10} by radiochemical techniques. In an in-pile experiment at Brookhaven National Laboratory, Upton, N.Y.[3] the solubility was observed to be 8×10^{-7} mole fraction under the same conditions. The wide scatter of the experimental data attests to the experimental difficulty inherent in measuring such low concentrations with any accuracy. Additional complica-

TABLE 1

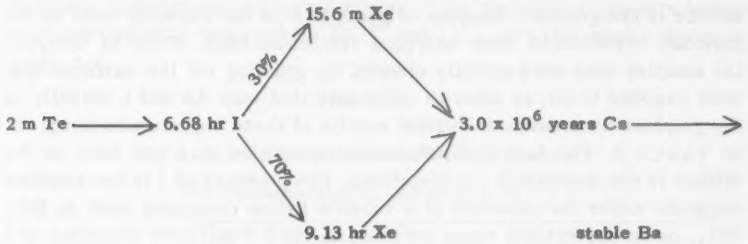
Fission product	*Yield, atoms per 100 fissions*	*Cross section, barns*
Xe^{135}	7.15	2.7×10^6
Sm^{149}	0.70	40,800
Sm^{151}	0.26	10,000
Gd^{157}	0.01	240,000
Nd^{143}	4.55	324
Cd^{113}	0.02	20,000

tions arise from adsorption effects that are of the same order of magnitude as the solubilities even in very carefully designed experiments.

In view of the considerable differences in the results cited above and in view of the importance of the problem to the design of an LMFR, it was decided to measure the solubility of Xe in Bi under conditions that would reduce the errors caused by surface adsorption effects. The experiments consisted of equilibration of Xe containing active Xe^{133} at a known pressure with U-Bi fuel for periods of 75 to 150 hours. Vigorous stirring is provided during the entire equilibration period to ensure a more rapid approach to equilibrium. When the equilibration period is completed, stirring is discontinued, and a period of three hours elapses before removal of samples in order to permit the escape of Xe mechanically trapped in the U-Bi fuel during the equilibration period. Samples of both metal and gas phases are analyzed radiochemically. The sampling of the metal phase is carried out under conditions that are believed to minimize errors due to adsorption of Xe on the surfaces of the polished metal sampling cup. The average of 14 such measurements at 540° C. was 2.4×10^{-9} moles/cm.^{3}-atm. Using the temperature correction of McMillan[4] this would become 1.2×10^{-9} moles/cm.^{3}-atm. at 500° C., or 2.5×10^{-8} moles Xe/mole Bi-atm.

A theoretical calculation by McMillan[4] has shown that at equilibrium a sphere of liquid Bi suspended in an atmosphere of xenon will contain about 10^8 atoms of Xe adsorbed on the gas-liquid interface for every atom of Xe dissolved in the body of the sphere. This tendency of Xe to concentrate at gas-liquid interfaces is a potentially serious problem for an LMFR because the U-Bi fuel does not wet the graphite walls of the fuel channels. The degree to which Xe tends to concentrate at such an interface was studied in a series of experiments in which graphite capsules (12 mm. o.d., 9 mm. i.d., 100 mm. high) were filled with U-Bi fuel and irradiated in the BNL pile for periods of 15 days at an average flux of 2×10^{12} neutrons/cm.^{2}-sec. In some of the experiments irradiation was at 500° C.

(that is, the U-Bi fuel was in a molten state), while in other experiments the irradiations were performed on solid fuel and followed by equilibration at 500° C. for periods of time ranging from a few minutes to 150 hours. After equilibration, samples of Bi from the central portion of the capsule and from the Bi adjacent to and including the interface were removed and analyzed radiochemically for I^{131} and Xe^{133}. The interest in I stems from the fact that it is the precursor of Xe in the 135 series and the distribution of Xe^{135} in the core will be dependent to a large extent on the



distribution of its precursor, the 6.68 hour I^{135}. Numerous experiments of the type described above have confirmed the validity of the McMillan calculation. A typical set of results is given in TABLE 2. These results show that both Xe and I have a strong tendency to concentrate at or near

TABLE 2

XENON AND IODINE DISTRIBUTION IN CAPSULES FILLED WITH U-BI FUEL

[All concentrations in (atoms/g) $\times 10^{-10}$]

	Xe^{133}		I^{131}	
	In bulk Bi	*In peripheral Bi*	*In bulk Bi*	*In peripheral Bi*
	0.16	2.3	0.10	20.0
(a)	0.22	1.8	0.13	13.2
	0.21	1.6	0.25	11.2
	0.17	219	3.31	1440
(b)	0.10	169	2.76	1380
	0.62	347	3.24	2440

(a) Capsule G-020: irradiated for 39 hours at 1.33×10^{12} neuts cm.^{2}-sec. in solid state. Equilibrated for 75 hours at 500° C. 1000 ppm U^{238}.

(b) Capsule G-180: irradiated at 500° C. for 15 days at 2×10^{12} neuts/cm.^{2}-sec.; 1000 ppm U^{235}.

the liquid metal-graphite boundary. The measured ratios of the concentrations in the peripheral Bi to the bulk Bi are much lower than the calculated 10^8 for the simple reason that it is impossible to isolate a true surface for analysis. The concentration of fission products is shown in FIGURE 1, which is a radiograph of a cross section of capsule G-180. The ring of intense activity is due probably in part to nuclides other than Xe and I, although the diffuse activity in the graphite is strongly suggestive of volatile nuclides, such as Xe, Kr, I, and Br.

The next problem of importance is that of the actual penetration of I and Xe in the graphite. Samples of graphite from the capsules used in the previous experiments were analyzed radiochemically. Prior to analysis the samples were mechanically cleaned by grinding off the surfaces that were exposed to Bi, in order to make sure that only Xe and I actually in the graphite are measured. Typical results of these experiments are given in TABLE 3. The fact that substantial quantities of a gas such as Xe diffuse in the graphite is not surprising. The presence of I in the graphite suggests either the presence of a volatile iodine compound such as BiI_3 PoI_3, or an appreciable vapor pressure of the I itself over solutions of I in Bi at 500°C.

The presence of iodine and xenon in the graphite in concentrations per unit volume that are of the order of, and in some cases even higher than, the concentrations in the fuel could be a serious problem in all reactors in which a graphite core is in direct contact with a fluid fuel, not only from the point of view of neutron poisoning, but also from the standpoint of reactor safety. Since one is primarily concerned with the 6.68 hour I

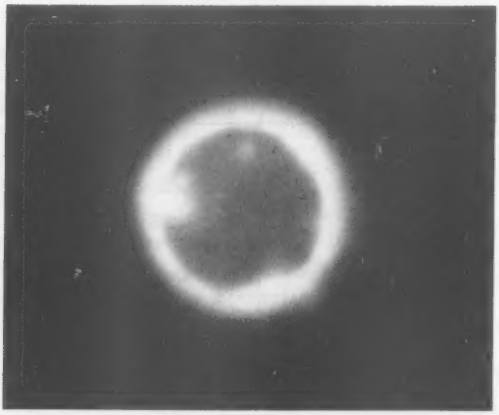


FIGURE 1. Accumulation of fission products on liquid metal-graphite boundary.

and the 9.13 hour Xe, the question of the rate at which these nuclides penetrate the graphite is of importance. Thus, the penetration rates may be so low as to result in the decay of these nuclides before any substantial concentrations are attained in the graphite. To obtain quantitative information on this aspect of the problem, 3 special capsules consisting of a U-Bi core (9 mm. diameter) surrounded by 3 concentric graphite layers of 1.5-mm. thickness each have been made of Graphitite "G", with all surfaces precision machined for a very tight fit. These capsules were irradiated for 15 days in the BNL pile and then equilibrated at 500° C. for 75 hours, 15 hours, and 5 hours. Samples of the successive graphite layers were subsequently analyzed for Xe^{133} and I^{131}, with the results shown in TABLE 4.

TABLE 3

PENETRATION OF XE AND I IN GRAPHITE

[All concentrations in (atoms/g) $\times 10^{-10}$]

Capsule	Xe^{133}	I^{131}	*Remarks*
G-160	3.1	44.4	1000 ppm. U^{238}; molten-state irradiation for 15 days
	1.4	46.4	
	1.6	29.6	
G-220	440	3200	1000 ppm. U^{235}; molten-state irradiation for 15 days
	91 to 0	6800	
	560.0	---	

TABLE 4*

RATES OF PENETRATION OF XE AND I IN GRAPHITITE "G" AT 500° C.

[All concentrations in (atoms/g) $\times 10^{-10}$]

Equilibration time (hours)	*In first layer*	*In second layer*	*In third layer*
75 hours Xe^{133}	0.62	0.27	0.32
I^{131}	4.1	5.0	3.2
15 hours Xe^{133}	0.21	0.15	0.06
I^{131}	12.0	18.5	3.3
5 hours Xe^{133}	4.0	0.78	0.83
I^{131}	121.0	14.3	5.8

*All figures are averages of results obtained in analyzing three samples.

It is seen that 75-hour equilibration results in a uniform distribution throughout the three layers of graphite (the difference of 0.62×10^{10} and 0.27×10^{10} is not statistically significant). Significant differences in the second and third layers become noticeable at 15-hour equilibration. When the equilibration period is shortened further to 5 hours, a concentration difference is noted also between the first and second layers. FIGURE 2 shows an autoradiograph obtained in the 75-hour equilibration experiments. The high degree of activity accumulated on the inner surface and the penetration of all 3 layers by radioactive materials are evident.

The results presented in TABLE 4 make possible an approximate calculation of the rate at which Xe and I concentrations will build up in an LMFR core for various graphite-to-fuel ratios.

Because of the low solubility of Th in Bi, an LMFR may become a thermal breeder only by introducing a suitable Th compound as a suspension in the liquid Bi. One possible compound that could be used for such a purpose is ThO_2. One of the important questions related to the use of ThO_2 slurries in an LMFR is that of the readsorption of fission gases on the suspended particles. This would make the gases unavailable for removal at the degasser and thus make difficult the maintenance of a low Xe^{135} poisoning level in the reactor. Work in the Netherlands on an aqueous suspension of UO_2 has shown that such readsorption of fission gases on the particles does occur and constitutes a serious problem in such slurry systems.

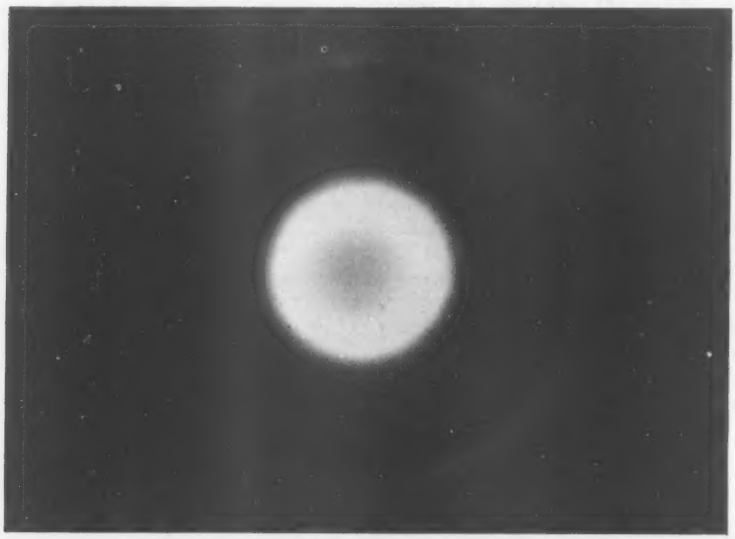


FIGURE 2. Diffusion of volatile fission products through graphite.

In order to measure the extent of this effect, a sample made up of 5 gm. of ThO_2 suspended in 49 gm. of Bi with 0.5 wt. percentage of Mg as an additive was irradiated at 500° C. for 5 months in the Brookhaven National Laboratory pile. The ThO_2 particles, about 4 μ in size, were obtained by crushing fused thoria.

After irradiation, the capsule was sectioned in such a way that samples could be obtained from the top ("T" samples) and from the bottom of the ingot "B" samples). It was thought that the 5 months' irradiation without motion of any kind would bring about a concentration of the particles in the top samples, and this should bring about higher Xe and I concentrations in these samples if there were a tendency for these nuclides to be adsorbed on the particles.

Photomicrographs of the "B" and "T" samples revealed, however, that there was no difference in the concentration of ThO_2, that is, that no segregation had taken place during the irradiation. This was confirmed by chemical analyses that gave the concentration of ThO_2 in the "B" sample as 4.74 per cent and in the "T" sample as 6.13 per cent. The Xe concentrations in the "B," the "T," and the graphite samples are shown in TABLE 5. For the purposes of comparison, this table contains typical results obtained in a U-Bi solution fuel capsule.

TABLE 5

XENON ADSORPTION ON ThO_2 PARTICLES

(All concentrations in atoms/g)

Xe^{131} concentrations in ThO_2-Bi slurry capsule		*Xe^{131} concentrations in solution fuel capsule*	
Top sample	1.0×10^{11}	In bulk:	1.0×10^{9}
Bottom sample	1.1×10^{11}	In peripheral Bi	6.1×10^{10}
Graphite:	3.2×10^{9}		
Graphite:	4.2×10^{9}		
Graphite:	0.8×10^{9}		
Av. graphite:	2.7×10^{9}	In graphite:	1.5×10^{11}

Comparison between the top and bottom samples shows that there is no difference between the two, as expected on the basis of the uniformity of the ThO_2 distribution. In both samples, however, the Xe concentration is much more than what one would expect to find in the bulk of the Bi if there were no ThO_2 particles present. This is suggestive of substantial adsorption of Xe on the particles. Further evidence for this is to be found

in the comparison of the average Xe concentration in the graphite of the slurry capsule (2.7×10^9) with the much higher (1.5×10^{11}) concentration found in the graphite of the solution fuel capsule. In the former the Xe is apparently absorbed preferentially on the ThO_2 particles.

Another portion of the irradiated slurry alloy was melted in a closed tube and filtered through a fine frit that retained the ThO_2 particles together with a small portion of the Bi, while most of the Bi was filtered through. The Xe^{131} activity liberated during the melting process was measured, as were the Xe^{131} activities in the filtrate and the residue. These activities were as follows: (1) Xe^{131} liberated during melting of 1.85 g. of alloy: 1.87×10^4 d/m; (2) Xe^{131} in 0.55 g. of Bi filtrate: 7.43×10^2 d/m; (3) Xe^{131} in 0.13 g. of ThO_2 + 0.59 g. Bi: 1.58×10^4 d/m.

Comparing the third and second activities, it is obvious that 95 per cent of the Xe^{131} measured in the alloy is associated with the ThO^2. This is in full agreement with the results presented in TABLE 5.

Thus, in the light of present evidence, it appears that the major problem in reducing nonproductive neutron capture by Xe^{135} in a liquid metal fuel reactor, and probably in other homogeneous reactors, is the adsorption of Xe and its precursor, I, on surfaces within the core of the reactor.

Nonvolatile Fission Products[5-6]

The nonvolatile fission products comprise a large number of elements of widely varying chemical and physical properties, and their removal poses an even more complex problem than does the removal of the volatile fission products. It is extremely desirable to remove them by processes that do not require the conversion of the bulk of the bismuth to some other form, such as an aqueous solution. Accordingly, a reagent that would selectively extract fission products from the melt without affecting the bismuth was sought very early. It was found that certain mixtures of fused chlorides served the purpose well. They were entirely immiscible with, and inert toward, the Bi; but several of the most important fission products transferred almost quantitatively into the salt phase. Analysis of the distribution coefficients under various conditions suggested that the process was chemical rather than physical, and involved converting the fission products to chlorides. Thus, if $MgCl_2$ is the extracting salt and La is taken as a representative fission product, there is set up the equilibrium:

$$3MgCl_2 + 2\ La\ (in\ Bi) \rightleftharpoons 3Mg\ (in\ Bi) + 2\ LaCl_3 \qquad (1)$$

The left-to-right reaction goes nearly to completion, and the $LaCl_3$ formed dissolves in the excess of liquid $MgCl_2$. A similar equilibrium can be written for the reaction between the uranium dissolved in the bismuth and the $MgCl_2$ but, in this case, the equilibrium constant is much smaller and

reaction ceases after a very small fraction of uranium has been chlorinated. The same holds true for many fission products. The criterion of extractability is, of course, the standard free-energy change involved in reactions of the type of EQUATION 1. The largest component of this quantity is the free energy of formation of the chlorides involved; thus, the rough prediction can be made that those fission products whose chlorides are more stable, by this criterion, than $MgCl_2$ will be converted to their chlorides. This group consists of the members of columns 1A, 2A, and 3A of the Periodic Table, including the rare earths; the most important elements involved are Rb, Cs, Sr, Ba, La, Ce, Pr, Nd, Pm, Sm, Eu, and Gd. This group, while omitting many important fission products, does include the worst neutron absorbers.

Experiments on the partition of elements, taken singly, between salt and metal phases confirmed the fact that those listed do indeed transfer from metal to salt, and that those with the most stable chlorides transfer most completely.

The equilibrium constant for reaction (1) may be written in the usual way as

$$K = \frac{[LaCl_3]^2\ [Mg]^3}{[La]^2\ [MgCl_2]^3} = e^{-\Delta F/RT} \qquad (2)$$

That is, the ratio of $[LaCl_3]$, the activity of salt-phase La, to [La], the activity of Bi-phase La, is a constant if the [Mg] and $[MgCl_2]$, or their ratio, are held constant. In practice, these latter activities or concentrations do remain sensibly constant at any one stage of an extraction process, since they are initially very much greater than the fission-product concentrations. It is convenient, both in experiment and calculation, to take the $[Mg]/[MgCl_2]$ ratio as the independent variable and study the dependence on it of the distribution of the various fission products. To define this ratio is to define an oxidation potential. Each fission product may be considered to have a characteristic standard oxidation potential in the Bi-salt system when present in each phase at some arbitrarily chosen standard concentration. Its distribution behavior in a particular salt-metal system can then be expressed simply in terms of the difference between its standard potential and the existing potential in that system.

A few elements were selected for a more thorough study with the aim of testing the constancy of the equilibrium constants and, if possible, correlating these with the thermodynamic properties of the compounds and mixtures involved.

The experimental results are most conveniently shown in graphs of the logarithm of the distribution coefficient of a solute, k = (concentration in salt)/(concentration in metal), versus the logarithm of the Mg concentration in the Bi expressed as parts per million by weight, ppm (the linear scale across the top of FIGURE 3 shows the oxidation-reduction potential

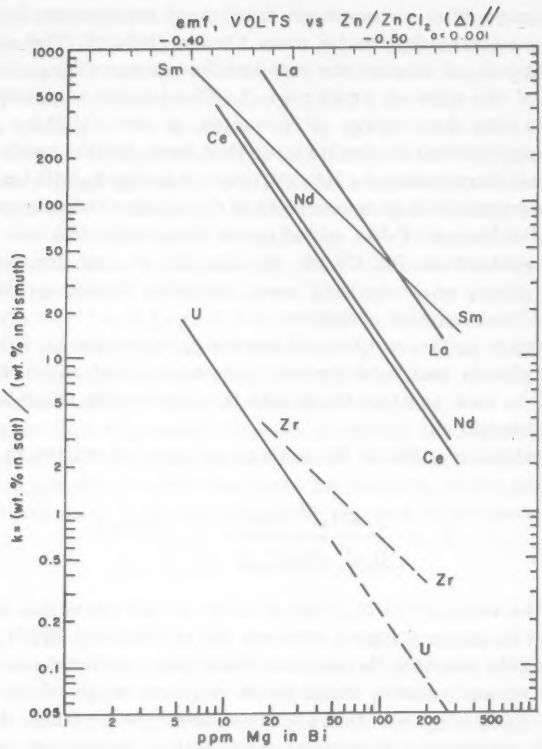


FIGURE 3. The distribution of solutes between $MgCl_2$-NaCl-KCl and Mg-Bi.

measured against a reference electrode). FIGURE 3 thus represents our best experimental results on Ce, La, Nd, Sm, Zr, and U. The straight lines can be justified both as to linearity and slope by an analysis of the chemical equilibria involved. From a practical point of view, we are interested in the separation factor, which is the ratio of the distribution coefficient of a fission product element to that of U. This value is represented by the vertical separation between lines in FIGURE 3, and has a value of 50 or more for Ce versus U, which is ample for a separation process.

It should be possible to predict the value of a separation factor, given chloride free energies and the other requisite thermodynamic data: the activity coefficients of Mg and the fission product species in Bi and of the chlorides of these elements in the salt phase. These were unknown, and had to be determined experimentally. The most convenient way of obtaining these functions is from the electromotive force of suitably

designed galvanic cells. A number of such cells have been measured. For metal systems, the cells were of the form

$$M\ (s \text{ or } \ell)/M^{n+} \text{ in supporting electrolyte}/M\text{-Bi}\ (\ell)$$

where M is a metal more electropositive than Bi, M^{n+} is an ion of metal M of valence n, M-Bi is a Bi alloy of the metal, the supporting electrolyte is an inert molten salt, and (s) and (ℓ) refer to the solid and liquid states, respectively.

The EMF of such a cell is related to the partial molar free energy of mixing and the activity of metal M by

$$F_M = -n\mathcal{F}E = RT\ell n\, a_M = RT\ell n\, f_M N_M$$

where F_M is the partial molar free energy of mixing; $\mathcal{F}$, the Faraday constant; E, the electromotive force; T, the absolute temperature; a_M, the activity of the metal; f_M, its activity coefficient; and N_M its mole fraction.

Solutions of the following metals in bismuth were examined by means of such cells: Pb, Ce, Mg, U, Li, Na, and Zr. In most of the cells, the composition of the alloy electrode was varied over a small concentration range in the dilute region appropriate to LMFR systems. Most fission products in an LMFR do not reach a concentration where their activity coefficients have a value different from that at infinite dilution, that is, they are in the region where Henry's Law is obeyed. Some of the infinite-dilution values are given in TABLE 6.

TABLE 6

System	*Temperature (°C.)*	f_M
Ce-Bi	500	3×10^{-14}
Mg-Bi	500	2×10^{-3}
U-Bi	500	1×10^{-5}
Li-Bi	450	1×10^{-5}
Na-Bi	500	8.5×10^{-5}
Zr-Bi	700	7×10^{-4}

In the cells used for the study of salt mixtures, the composition of the electrolyte rather than of an electrode was varied. A typical cell arrangement was the following:

$$Mg/MgCl_2\ (x),\ KCl\ (1-x)/Cl_2\ (\text{graphite})$$

Its EMF is a measure of the free-energy change in the reaction

$$Mg + Cl_2 \longrightarrow MgCl_2 \text{ in } MgCl_2\,(x),\ KCl\,(1-x)$$

Its value E is related to the free energy of formation of $MgCl_2$ in its standard state, ΔF_0, and its activity in the mixture, a_{MgCl_2} by the expression

$$E = -\frac{\Delta F}{n\mathcal{F}} = -\frac{1}{n\mathcal{F}}\,(\Delta F_0 + RT\ln a_{MgCl_2})$$

With Mg or Mg-Bi as electrode, the following salt systems were investigated, the concentration of $MgCl_2$ in each being varied from 0 to 100 per cent:

$$MgCl_2 - KCl$$
$$MgCl_2 - (NaCl + KCl)$$
$$MgCl_2 - NaCl$$

In the second system, the NaCl/KCl molar ratio was kept constant at 1.043; this is their ratio in the binary eutectic mixture. With a Ce-Bi electrode, $CeCl_3$-KCl electrolytes were investigated. Strong negative deviations from Raoult's law were found in all cases.

Of those fission products that are not removable from U-Bi by fused-salt treatment, the most important are Ru, Rh, Pd, Mo, Te, and Tc. It has been found that several of these can be removed by application of a modified Parkes process; that is, a small quantity of zinc is added, which forms intermetallic compounds with Ru, Rh, Pd, and Te, and possibly others. These become insoluble as the temperature is lowered, and can be filtered out or skimmed off.[7]

To summarize: it is hoped that the major fission products can be removed by the three operations, outgassing, salt contacting, and zinc treatment. The decontamination efficiency may be reduced by the adsorption of some elements on solid surfaces.

Acknowledgment

The experimental results described in this paper were not produced solely by us, but by the cooperative effort of a large number of investigators, members of the Nuclear Engineering Department of the Brookhaven National Laboratory.

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DIVISION OF PSYCHOLOGY

ON THE ORIGIN OF THE REPRODUCTIVE BEHAVIOR CYCLE IN DOVES*

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Introduction

The ring dove (*Streptopelia risoria*) is a brownish bird about the size of a robin, which in its shape, voice, and general movements resembles the much larger common pigeon (*Columba livia*), to which it is closely related. It exists as a domestic bird in many parts of the world, and breeds well in the laboratory. In our laboratory, my associates and I breed these birds in pairs in cages approximately 33 in. long, 18 in. deep, and 14 in. high. In these cages, in artificially lighted rooms in which the day (light period) remains 14 hours long and in which the temperature remains constant at approximately 75 degrees F. the year round, ring doves breed well at any time of the year.

The reproductive behavior cycle. If a pair of ring doves that have never previously met each other but have both had successful breeding experience, rearing one or more broods of young, is placed together in a breeding cage, both birds show a regular, predictable, and very striking series of changes in behavior. For the first day or two, the predominant behavior will consist of courtship, in which the male makes stereotyped bowing movements toward the female, accompanied by a characteristic cooing vocalization (other aspects of the preliminary courtship need not concern us here). Starting on the second or third day, the predominant activity appears to be nest-building behavior. The nest is usually built in a glass bowl provided by the experimenter, and the typical pattern is for a male to pick up most of the nesting material (pine needles) and carry it to the nest, where the female does most of the actual building (Goodwin, 1955). After 3 or 4 days of this nest-building behavior, the behavior of the female suddenly changes. Whereas, during the early stages of nest building she could be easily frightened away from the nest by the hand of the experimenter thrust into the cage, she is now seen to stand firmly on the nest and to be very difficult to dislodge, even going so far as to strike with her wing at the intruding hand. At this time she remains continuously on the nest, leaving it only to feed. The onset of this behavior usually means that the female is about to

*This paper was presented at a meeting of the Division on May 18, 1959. Most of the experiments reported in this article are described in greater detail elsewhere (Lehrman, 1958 and 1958a).

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lay an egg. Her first egg will be laid at about 5 o'clock in the afternoon, from 1 to 3 days after the onset of this firm attachment to the nest. The second egg will be laid approximately 40 hours later, at about 9 o'clock on the second morning following the first egg. Some time during the period between the laying of the first and second eggs, the female will start to incubate the egg. The incubation behavior consists of stepping very gingerly on the nest and slowly settling down on the egg, simultaneously erecting the feathers on the lower abdomen so as to bare a naked, unfeathered ventral area, which is pressed directly against the egg; the blood vessels in this area provide the heat for incubation. After the second egg is laid, the male and female take turns incubating the eggs, the male usually sitting for about 6 hours in the middle of the day, the female for the remaining 18 hours. The eggs hatch after about 14 days of incubation, and both parents take turns brooding the young and feeding them, at first by regurgitating a substance (crop milk), which is produced in the bird's crop, later by regurgitating grain. By the time the young are about 20 days of age, they are substantially able to feed themselves, and the parents become increasingly reluctant to feed them. At about this time, courtship again becomes prominent in the behavior of the adults, and the entire cycle starts again.

We thus have a reproductive behavior cycle that is repeated at intervals of about 6 to 7 weeks and consists of a series of striking changes in the predominant behavior characterizing the birds' activities. These changes are not merely due to changes in the external circumstances, but reflect internal changes going on in the birds. Thus, the amounts of courtship and of nest building vary cyclically, in spite of the fact that the mate and the nesting material are continuously present. Birds offered young during the nest-building period will not accept them, nor will birds offered eggs during the courtship period show any interest in incubation. We must therefore conclude that these changes in behavior reflect internal physiological changes, undoubtedly associated with changes in endocrine secretion.

The physiological reproductive cycle. We can find in fact a series of regular anatomical changes that are associated with these behavioral changes and that are equally striking. For example, when the pairs are first placed in the breeding cage together (each bird having previously been in isolation for from 3 to 5 weeks), the oviduct of the female weighs approximately 800 mg. By the time she lays her first egg, 7 to 9 days later, the oviduct weighs perhaps 4000 mg., an increase of 500 or 600 per cent. The crop of each parent may weigh about 700 mg. when the birds are put together in the cage. When the eggs are laid a week later, the crops will not have increased in weight. However, after 2 weeks of incubation, the crops may weigh 3000 or 4000 mg., again an increase of 500 or 600 per cent. Changes in the weight of the liver,

the histology of the vas deferens and of the testes, and other such factors are equally striking.

It is apparent that both the behavioral and the anatomical changes taking place during the cycle are expressions of a basic cycle of physiological change that constitutes the reproductive cycle of these birds.

The problem. Although both birds of the pair show regular, cyclic changes in behavior and anatomy during a reproductive cycle, and although these changes are synchronized (so that, for example, the male is interested in sitting on eggs at about the same time that the female lays the eggs, and so that both birds become interested in feeding the young at about the same time), neither bird shows any such cyclicity when it is kept in isolation. A male kept alone in a cage remains permanently ready to court any bird placed in a cage with him, permanently uninterested in sitting on eggs, or in feeding young, or in building a nest. A female placed alone in a cage does not lay eggs at regular intervals, and may lay no eggs at all.

It is apparent, then, that the cyclicity of reproductive behavior and physiology that is so characteristic of these birds under normal circumstances does not arise solely from internal physiological relationships within either the male or the female. The problem with which we wish to deal is that of the origin of this cyclicity.

Cyclic Development of Incubation Behavior

Induction of incubation behavior by external stimuli. If a pair of experienced ring doves is placed in a breeding cage provided with a supply of nesting material and with a nest bowl in which a nest with 2 eggs is already present, neither bird will sit on the eggs immediately. The birds will be found sitting on the eggs after from 4 to 7 days. During this period of 4 to 7 days the birds can be observed to go through the ordinary progression from courtship to nest building. Indeed, during the nest-building period, the birds could be seen actually standing on the nest containing the eggs, and building additional nests over the eggs!

Apparently the birds change, during a 4 to 7 day period, from birds that are not interested in sitting on eggs to birds that are interested in doing so. What stimulates this change? The first possibility is that the birds become habituated to the cage and that the latency of incubation behavior can be accounted for in this way. In order to test this possibility, we placed pairs of birds in breeding cages without any nesting material, nest bowls, or eggs, and separated the male from the female by an opaque partition. After 7 days the opaque partition was removed, and a nest bowl containing nest and eggs was placed in the cage. These birds, which had had 7 days to become accustomed to the cage, still took from 4 to 7 days before sitting on the eggs.

Another possibility is that the presence of the eggs induces the development of incubation behavior in the birds, but that it takes from 4 to 7 days for this effect to accumulate to a threshold value. In order to test this possibility, we placed pairs of birds in breeding cages each equipped with a nest bowl and with a supply of nesting material, but without any eggs. Seven days later we removed the nest bowls with the (partly built) nests, and replaced them with new nest bowls containing complete nests, each containing 2 eggs. All of these birds sat on the eggs on the first day after the eggs were introduced into the cages. Clearly, the presence of the eggs is not necessary for the development of whatever physiological changes underlie the onset of incubation behavior. However, when males or females were placed alone in breeding cages, each with a nest and 2 eggs, none of these birds sat on the eggs, although some of the observations were continued for as long as 6 weeks.

Clearly, stimuli arising from the presence of the mate and/or the nesting material induced the change from birds that are not interested in sitting on eggs to birds that are interested in doing so. A preliminary attempt to distinguish between the effects of stimuli from the mate and those of stimuli from the nesting material and/or nest bowl was made by the following procedure. Pairs of birds were placed in the breeding cages with no nest bowl or nesting material. After 7 days a nest bowl containing a nest with eggs was placed in each cage. Unlike the pairs which had had nesting material in the cage during their 7-day period of association with each other, none of these birds sat immediately. However, neither did they wait 4 days. All the birds were sitting on the eggs by the second day. During this one-day latency period the birds were seen to engage in intensive nest-building behavior.

We may conclude from these experiments that the birds in our various groups showed 3 different physiological-behavioral conditions: (1) courtship, (2) nest building, and (3) incubation behavior. Furthermore, under our experimental conditions, association with the mate (= participation in courtship?) facilitates the progression from readiness to court to readiness to build a nest. Furthermore, the availability of nesting material (= participation in nest building?) facilitates the progression from readiness to build a nest to readiness to sit on the eggs. In other words, stimuli coming from the mate foster one behavioral change, and stimuli associated with the presence of nesting material foster a further behavioral change. We may now ask what is the nature of these two successive changes in the condition of the birds.

Hormones and incubation behavior. It is a reasonable assumption that the changes induced by the various environmental conditions in the birds described thus far consist in the first place of changes in endocrine secretion. Our next step is to attempt to bring about the

changes described above by direct injection of hormones, rather than by exposure to the environmental conditions that, as we have shown, foster the physiological-behavioral changes.

Several pairs of birds with breeding experience were taken from isolation cages and placed in pairs in the breeding cages, as in the experiments previously described, except that, starting one week before the birds were due to be placed in the experimental cages, each bird was given daily injections of progesterone. After 7 such injections, the birds were placed in the experimental cages, with a nest bowl supplied with nest and eggs. All of these birds sat on the eggs within 2 hours, some of them within 5 or 6 minutes. (Note that birds untreated with progesterone, but otherwise handled in exactly this way, would not sit on the eggs until after some 4 to 7 days, during which they would go through courtship and nest-building phases).

When the hormone treatment was with estrogenic hormone instead of progesterone, the birds did not sit on the eggs immediately. However, neither did they wait 4 days. Most of these birds sat after a period of 1 to 3 days, during which all the birds were engaged in intensive nest-building behavior.

Testosterone does not induce incubation behavior.

The experiments thus far described indicate that stimuli arising from participation in courtship stimulate the secretion of estrogen, which in turn induces nest-building behavior. Furthermore, under these conditions, it seems likely that participation in nest-building behavior (or stimuli arising from the presence of the nesting material) stimulates the secretion of progesterone which, in turn, induces incubation behavior. Autopsy data on these birds, which cannot be reported here, and studies of the anatomical effects of these hormones and of the anatomical changes during the cycle, support these conclusions (Lehrman, 1958 and 1958a; Lehrman and Brody, 1957).

From Incubation to Care of the Young

Patel (1936) has shown that stimuli arising from the act of incubation (in which both male and female share) stimulate the secretion of prolactin by the pituitary gland which, in turn, causes the development of the crop so that it is capable of secreting crop milk for the young. When pigeons were allowed to sit on their eggs, the crop developed very rapidly, so that it increased in weight by 600 per cent by the time the eggs were hatched (it is known that crop development occurs only under the influence of prolactin secreted by the pituitary gland [Riddle and Bates, 1933]). When Patel removed a pigeon from the breeding cage just after the beginning of incubation and placed him in another room, the crop did not develop. If the removal was effected after the crop had already begun to develop, it immediately regressed to its

resting state. When, however, a male pigeon was removed from the breeding cage and placed in an adjacent cage from which he could see his mate sitting upon the eggs, his crop developed just as if he were sitting on the eggs himself. This clearly indicates that stimuli associated with incubation (including visual stimuli) actually induced the secretion of prolactin by the pituitary gland. This prolactin, in turn, induces the physiological changes underlying the transition from incubation behavior to the behavior of brooding and feeding the young (Lehrman, 1955).

It has been demonstrated further, in various species of birds, although not yet in doves, that the presence of the young inhibits the secretion of the hormones responsible for renewed courtship and nest building. Removal of the young before they normally would leave the nest may cause the parents to engage in renewed sexual activity and nest building earlier than expected (cf. Lehrman, 1959).

The Physiological Link Between Environment and Endocrine System

It is apparent from the experiments described thus far that stimuli from the external environment are capable of causing or stimulating changes in the pattern of endocrine secretion. This means, of course, in the first place, pituitary secretion, since secretory activity of the gonads is dependent upon stimulation by pituitary hormones.

Recent physiological work makes it abundantly clear that the activity of the pituitary gland is controlled in considerable detail by stimuli (or rather by stimulating substances) reaching it from the hypothalamus (Harris, 1955). This has been convincingly shown in the case of most of the pituitary hormones, including specifically the gonadotropic hormones. There exists, therefore, a well-demonstrated physiological-anatomical mechanism on the basis of which it is possible for hormonal secretions to occur reflexly in response to external stimuli of the kind that we usually think of as eliciting behavioral responses.

Conclusion

It is now possible to give a general or schematic answer to the question raised at the beginning of our discussion: What is the origin of the cyclicity of changes in behavior and in the reproductive system in doves kept in pairs, when such cyclicity does not occur in either bird kept in isolation?

The progression of behavior and physiology from one stage to the next and the synchronization of the cycles of the two members of the pair appear to be accomplished through the fact that the presence and behavior of one member of the pair are capable of providing stimuli that cause changes in endocrine secretion in the other member, and that this is a reciprocal effect. There are two reciprocal relationships involved:

first, members of the pair, by their presence, activity, and the products of their activity, reciprocally influence each others' pituitary glands so as to cause changes in physiological state; and, second, there is a reciprocal relationship between the effects of hormones upon behavior patterns and behavioral tendencies on the one hand, and, on the other, the effects of stimuli arising from the behavior (which may have been induced by hormone secretion) upon the secretion of the hormones themselves.

The cause of the behavior cycles must therefore be sought in the fact that the endocrine system is an effector system that is capable of responding to external stimuli mediated by the nervous system and is in turn capable of changing the behavior of the animal.

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DIVISION OF INSTRUMENTATION

ELECTRONIC COMMAND OF MOVEMENT AND BEHAVIOR*

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Modern technology has created instruments able to stimulate electrically any cerebral structure in the fully conscious subject. Electricity influences the neurons, can direct the brain to produce movements, hallucinations, drives, emotions, hostility, and friendliness, and is even able to modify human thoughts. Thus electronic instruments can control functions of the same organ that invented them, closing, we could say, a circle of direct relation between intellectual and material worlds. Cerebral research creates a situation unique in science. We must reason to discover the basis of reason, construct theories on how theories are constructed, and use the brain to understand brain functions. Presentation of experimental data, with discussion of possibilities and limits of electronic control of cerebral functions, is the purpose of this paper.

Advances in Methodology

Direct study of the brain by penetration of the several layers of scalp, bone, aponeurosis, and membranes which shelter the cerebrum had to wait upon the development of anesthesia and surgery. Anesthesia, however, was a serious handicap because it blocked the most important cerebral functions, and methods were necessary for painless exploration of the brain in conscious subjects. Also, it has been difficult to relate psychological concepts such as drives and emotions to physiological data, and investigators in both disciplines for a long time spoke different languages. Fortunately, bridging efforts have been successful, as proved by the growing interest in physiological psychology. In the last ten years methods begun in Europe (Hess, 1932) have been further developed in this country (Sheer, 1959), and today with implanted electrodes it is possible to record cerebral electric activity or to send electric stimulations into the brain of conscious animals or humans (Delgado, 1955; Delgado and Hamlin, 1956).

In our studies, begun ten years ago, bundles of insulated wires as fine as hairs are introduced into the brain. Location of each contact is preselected in stereotaxic maps of the brain (Bailey and Schaltenbrand,

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in press; Jasper and Ajmone-Marsan; Olszewski, 1952), and the leads are placed with a micromanipulator through a small hole drilled in the skull, and are cemented to the bone. The terminal socket remains outside the scalp (FIGURE 1) to establish connections with the instruments. Several electrode assemblies with a total of as many as 42 contacts have been implanted in a single brain. A few days after implantation the effect of anesthesia has disappeared, the operatory wound has healed, and study of the unexposed brain in fully conscious subjects can begin and be repeated for months or years.

For some investigations the presence of connecting leads is impracticable because they obstruct freedom of movement or cause inhibitions; wireless stimulation of the brain therefore became desirable. To solve this problem a very small transistor stimulator and a tiny timing mechanism that programs the instant and the rhythm of the stimulations was constructed (Delgado, 1959*a*). Radio transmission is not needed and the whole instrument is small enough to be carried by an animal on a collar. Intermittent or uninterrupted long-term stimulations for hours or days are thus made possible.

To analyze the results of brain stimulation, especially their effect on social activities, a method for continuous recording of behavior was developed. A colony of animals (8 cats in some studies, 6 monkeys in

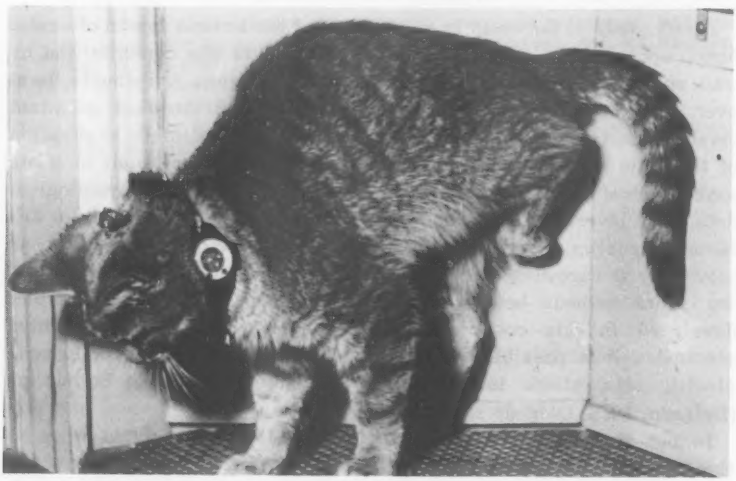

FIGURE 1. Three assemblies of 7 electrodes each have been implanted in the brain. The 3 terminal sockets are shown at the back of the head. A transistor-timed stimulator is carried on the collar for wireless stimulation of the brain. Flexion of the left leg is evoked by stimulation of the right motor cortex. There is no emotional disturbance, and the animal's posture is adapted to the performance of the evoked movement.

another) is permanently housed in a large cage with a glass front in an air-conditioned soundproof room. A 16-mm. motion picture camera with a time-lapse mechanism takes between 4,000 and 20,000 pictures daily according to the experiment, providing sufficient data for statistical evaluation of social relations. The films are analyzed with the aid of an automatic projector, and an electric typewriter equipped with electronic counters quantifies the behavior with speed and accuracy. Studies are made on physiological, psychological, and sociological levels, paying special attention to correlations among them.

Motor Activity

The fact that electric stimulation of the cerebral cortex could produce movement was known many years ago, but the movements were described as crude – lacking finesse, integration, and purpose. Methodological handicaps such as trauma, exposed brain, and use of anesthesia probably disturbed the physiology of the neurons and was responsible for the elementary type of responses obtained. Recent exploration of the motor cortex with implanted electrodes in unanesthetized subjects has proved that effects similar to spontaneous activity could be induced by brain excitation (Delgado, 1952). For example, a cat would raise its left hindleg slowly, the other limbs and the body adapting themselves to proper performance of the movement (FIGURE 1). When the same cerebral point was stimulated with the animal in a different starting position other groups of muscles would be involved; also, obstacles placed in the path of the movement would be avoided, but the final posture would be similar in all stimulations of the same point.

A great variety of activities has been evoked by brain stimulation, such as yawning, in the cat; wagging the tail, in the dog; winking, in the monkey; and turning the head, in humans. A complete list of evoked movements would include a great number of motor activities observed in normal life. Often the animals resembled electric toys, having predictable series of movements controlled by pushbuttons. These experiments did not appear to be uncomfortable; the cats especially seemed to enjoy the attention paid to them and purred noisily during the studies. Sometimes there was a conflict of wills between experimental subject and scientist, and the animal tried to resist the evoked movement. However, if the intensity of stimulation was strong enough, the electronic command always prevailed against the animal's opposition.

Summation of spontaneous and evoked movements was demonstrated, showing that artificial excitation of the brain could interact with its normal function. A hungry cat raised its paw to take food; mild stimulation of the sulcus presylvius in the same animal evoked raising of the foreleg. When food was offered and the sulcus presylvius was stimulated at the same time, an exaggerated raising of the paw followed, and the animal overestimated the movement necessary to reach the food. Monkeys

proved to be resourceful in difficult situations. Extension of both arms was evoked in one animal by stimulation of the cerebral cortex and, simultaneously, peanuts were offered. The monkey soon realized that it could no longer command its arms and began to take the peanuts with its feet.

Evoked motor effects did not cause emotional disturbance, modification of social behavior, or motor impairment, apart from that directly related to the evoked movement. Stimulation of the inferior part of the sulcus presylvius in a cat that was walking caused contractions of the face and winking of one eye without interrupting the animal's walk. Stimulation of the motor cortex in a monkey with the transistor collar is shown in FIGURE 2. While the animal was free in a colony, automatic excitations were delivered for 5 sec. in every minute for 1 hour. During all this time the animal continued normal social activities and, as shown in the figure, grooming was not interrupted in spite of facial contortions and contralateral turning of the head evoked by the stimulations.

Emotional Behavior

Rage has been evoked in cats by electric stimulation of the amygdaloid nucleus. Usually peaceful animals reacted to stimulation with piloerection, pupillary dilatation, aggressive snarling, clawing the air, and attacking any approaching object. Rage abated with the end of the stimulus; the cats were as friendly as before, and could be petted. Several

FIGURE 2. Electric excitation of the motor cortex with the transistor collar stimulator makes the monkey turn its head and wrinkle its face without interrupting social activities such as grooming.

investigators doubted that this type of response was true rage (Masserman, 1941), and suggested that it was a pseudoaffective reaction – the animal not being aware of it and therefore not emotionally involved. To test this we put two friendly cats together. When the amygdaloid nucleus was stimulated in one cat it immediately attacked the other fiercely and a fight ensued as shown in FIGURE 3, which ended with the end of the stimulus. After several experiments a state of mistrust existed between the animals. This experiment showed that the induced rage was not a sham reaction, but a well-oriented drive to attack and destroy.

Areas that inhibit or tame animals have been demonstrated in cats and particularly in monkeys that are normally wilder. Rhesus monkeys usually seize and bite any approaching object, but upon stimulation of the caudate nucleus the mouth closes, the face loses its aggressive expression, and the monkey relaxes and can safely be touched even on the mouth, as shown in FIGURE 4. Its usual ferocity resumes when stimulation is stopped.

Decrease of aggressiveness was also demonstrated in colonies. It is well known that one monkey always establishes itself as leader of a group, threatening, pushing away, and chasing the other members, which do not retaliate. In our experiments dominance was also manifested by the crowding together of the other monkeys in one corner,

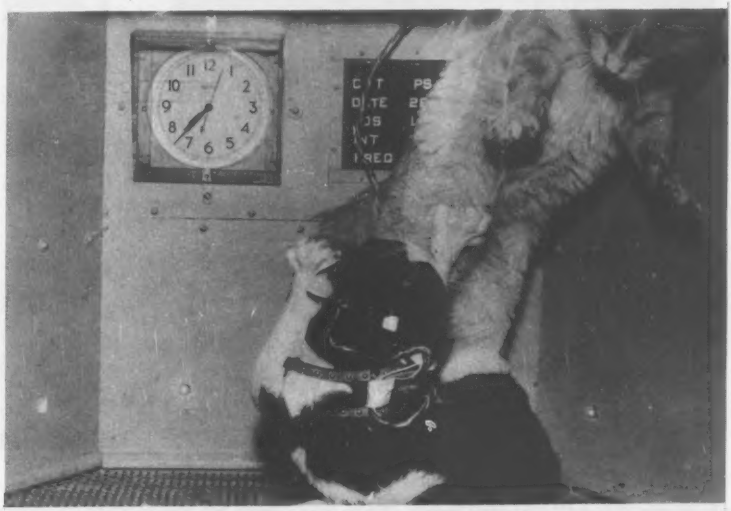

FIGURE 3. Electric stimulation of the tectal area in one of the cats evokes a fight; both cats show well-oriented aggressive and defensive movements. Pattern of evoked behavior is very often indistinguishable from spontaneous activity.

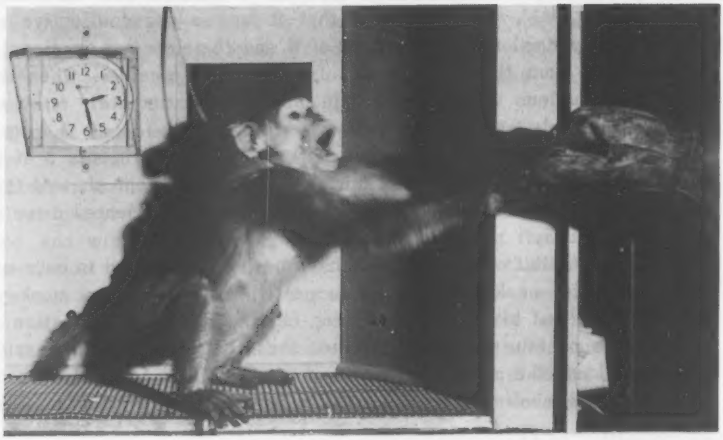

FIGURE 4. Rhesus monkeys are usually aggressive and, as shown in the upper control picture, it is dangerous to put a hand within reach of their teeth. Caudate nucleus stimulation tames the animal so that it is safe to touch its mouth. As soon as stimulation stops the monkey becomes aggressive again.

while the "boss" enjoyed most of the cage area by himself. By means of the transistor collar the boss was stimulated in the septal area; the resulting loss of aggressiveness was sensed immediately by the other monkeys, which then moved freely around the cage without any

fear of the former bully, who recovered his dominance, however, soon after stimulation was discontinued.

Learning of instrumental responses could be motivated by direct stimulation of the brain. To show this effect, cats were put into a glass cage and the intracerebral electrodes connected through long leads with a stimulator, allowing the animals freedom of movement. A buzzer sounded, followed two seconds later by automatic stimulation. The cats could prevent the stimulation by turning a wheel at one side of the cage. Excitation of some points of the brain did not modify the animals' behavior, but stimulation of tectum and lateral thalamus induced restlessness and attempts to hide or to escape. After several trials, by chance the cats turned the wheel stopping the stimulation. By trial and error the animals soon learned to turn the wheel as soon as the warning sound began, preventing the subsequent stimulation.

In another series of experiments cats and monkeys responded to acoustic or visual signals; they learned to press levers, to jump from one cage to another, to select one side of a testing table, and to perform a variety of instrumental responses, having as their only motivation the direct brain stimulation (Bursten and Delgado, 1958; Delgado *et al.*, 1954; 1956).

Stimulation of the Human Brain

Penfield and other investigators (Penfield and Japser, 1954) have described a variety of effects evoked by electric stimulation of the surgically exposed human brain. In recent years therapeutic implantation of electrodes in the brain of epileptics, schizophrenics, and other patients (Delgado and Hamlin, 1956) has provided a better opportunity to study the results of cerebral stimulation, for it could be repeated for several days in subjects who were in familiar and comfortable surroundings away from the operating room. In our studies the conversation of patient and therapist was rountinely tape-recorded and synchronized with simultaneous electroencephalographic recording and signals of stimulation. The patients were informed of the general procedure, but they were unaware that excitations or recordings were going on at any specific time. Blocking of thought was evoked by excitation of the frontal lobes. Stimulation (100 cycles/sec., 1 msec. of pulse duration, 10 volts for 5 sec.) was applied to one patient while he was reciting poetry, causing him to stop suddenly. After the stimulation the patient was asked what had happened, and he said that he had felt a funny sensation in his head and was unable to think of the words of the verse. He said, "My mind was blank, as if I had drunk a lot of beer." Reliability and specificity of the effect were demonstrated by sham stimulations alternated with true ones and by stimulations of other areas that failed to produce this effect. In another patient the

tape-recorded interview was later divided into periods of 2 minutes. In each period the number of seconds the patient spent in talking to the interviewer was quantified. Verbal expressions were also analyzed and classified according to 39 categories such as requests and demands, rejecting statements, friendly verbalization, and masculine striving. In this report (Higgins *et al.*, 1956) a significant increase in the amount of talking and in the number of friendly remarks followed electric stimulation of the inferolateral surface of the frontal lobes. Examples of the patient's friendly expressions were: "Can I say something in your ear? It is a little thing I forgot; happy valentine to you. Did you hear me?" In the same patient electric stimulation of the superior temporal convolution induced the appearance of feminine striving and confusion about his own sexual identity. These effects were specific, reliable, and statistically significant. For example, the patient, who was an 11-year-old boy, said, "I was thinking whether I was a boy or a girl, which one I'd like to be," and "I'd like to be a girl." After one of the stimulations the patient suddenly began to discuss his desire to get married, expressing then a wish to marry the male interviewer. In two adult female patients stimulation of the same region was also followed by discussion of marriage and expression of a wish to marry the therapist. Temporal-lobe stimulation produced in another patient open manifestations and declarations of pleasure, accompanied by giggles and joking with the therapist.

Fear has been caused by frontal-lobe stimulation. In one case, recorded in motion pictures, the patient during each excitation stopped her conversation and looked backwards, moving her head with an expression of deep anxiety. When asked the reason for her behavior, she replied that she felt some imminent threat of unknown nature.

The apparent spontaneity of these evoked effects should be emphasized. The fear, pleasure, giggles, friendliness, and doubt about sexual identity were expressed with words and behavior natural to the patient. The subjects did not feel that they were acting under any external force. However, other effects evoked by brain stimulation (hearing words, seeing a face, and moving the hand) were considered by the patients to be strange and detached from themselves.

Discussion

Data presented in this paper demonstrate that electric stimulation of specific areas of the brain could force an animal to perform complex acts indistinguishable from voluntary movements and could modify human behavior to the extent of producing fear or increasing friendliness and verbal output. Electric stimulation created impulses similar to physiological functions in that the evoked effects not only resembled spontaneous activities, but also combined or competed with them. Electronic

command overwhelmed the "will" of the experimental subject and always prevailed if sufficient intensity was applied. Many effects were evoked in our experiments, and it is conceivable that most of the somatic, autonomic, and physiological functions under cerebral control can be influenced by brain stimulation.

These facts would seem to support the distasteful conclusion that motion, emotion, and behavior can be directed by electric forces and that animals and humans can be controlled like robots by pushbuttons. If so, to the present collection of guided missiles a new one – the brain – could be added. However, the assumption of cerebral subordination to electronic command must be qualified. The probable role of electricity in brain stimulation is to activate, inhibit, or disturb the pre-existing physiological and psychological pattern, but it cannot create either of them. Motility and behavior depend upon neuronal correlations, including continuous integrations of sensory input that have great complexity in space and time. By stimulation we can initiate or influence these correlations, but electricity cannot be substituted for them. We may conceive of brain functions as a "play of forces" (Hess, 1957), a "balance" (Crosby, 1959), a state of "tuning" (Gellhorn, 1957), or as "antagonistic effects in continuous dynamic equilibrium" (Delgado, 1959*b*). Electric stimulation may shift the existing equilibrium, and the consequent imbalance may be directly observable, as in a motor response, or the stimulations may have a more subtle effect revealed only in an abnormal reaction to a normal stimulus. This subtle effect was seen in some amygdaloid stimulation of cats that were friendly and appeared unchanged during prolonged stimulation, but hissed, spat, and bit if we tried to pet them; their friendly behavior was recovered as soon as excitation ceased. It should be emphasized that we could predict increase in aggressiveness when specific areas were stimulated in any cat, but we could not predict the form in which the animal would express its evoked aggression. In other words, brain stimulation can elicit an emotional state, but it cannot change the basic characteristics of the experimental subject, and the pattern of the response (such as play, fighting, sex, and eating) will depend upon the individual.

The increased verbal output evoked by stimulation of the temporal lobe in humans also could be explained as a change in balance. The ideas and words used by the patients were the product of their own personalities, but their expression was facilitated (less inhibition? greater production?) by brain stimulation. In some cases the type of thought – friendly, antagonistic, sexual, and the idea of marriage – seemed to depend upon cerebral excitation, but even so it could not be said that the thoughts were created by electricity. More probably, the stimulation started a chain reaction among neurons that are responsible for

the complex phenomenon of thinking; the thoughts would be electrically initiated, but would depend upon experience, personality, and many other factors for their expression. We could probably compare some effects of brain stimulation with hormonal action, which may influence such factors as sexual drive and social behavior with the difference that cerebral excitation may be more powerful, faster, and more spectacular.

The use of brain stimulation in research has been very fruitful, but its therapeutical possibilities have barely been investigated. Problems of human application are certainly complex. However, it is reasonable to assume that electrically induced changes in cerebral balance could be useful in several illnesses in which mental imbalance is the main disturbance. The fact that some areas of the brain are infatigable (Delgado, 1959*b*), that it is possible to evoke lasting effects (Alonso-deFlorida and Delgado, 1958), and that a new technique for wireless stimulation of the brain has been developed (Delgado, 1959*a*), should contribute to influence the organ that influences us. At the same time it is important to establish the limits of electric stimulation of the brain in the hope that personality and our basic ideas are beyond electronic control.

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DIVISION OF BIOCHEMISTRY

THE PURIFICATION AND ACTION OF TUMOR FACTOR EXTRACTED FROM MOUSE AND HUMAN NEOPLASTIC TISSUE*

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The presence of a tumor factor** in Hodgkin's disease (HD), lymphomatous and neoplastic human tissue previously has not been demonstrated. To associate a tumor factor (TF) with the etiology of HD is difficult, since the disease has been observed only in man; and has not been induced in an experimental animal.

Believing that a more fundamental understanding of mammalian oncogenic factors might provide the rationale and techniques for studying human tumors and related syndromes, such as HD, the HD laboratories selected mouse neoplasia as a model system. The success achieved by Gross[1-3] in inducing leukemia and solid tumors in the C3H mouse and the confirmation of his results by others[4-7] suggested that crude cell-free filtrates of mouse leukemic tissue contained active TF. These crude cell-free filtrates, containing TF, are heterogeneous in that they contain a small amount of labile carcinogenic material along with enormous quantities of impurities.[8] It was hoped that the variability in activity, the lability, and the lack of reproducibility could be eliminated by the refinement of the crude extracts. Preliminary attempts to purify tumor-inducing substances from mouse leukemic tissue by standard viral purification techniques, such as cold methanol fractionation,[9] protamine precipitation,[10] and genitron homogenization[11] resulted in preparations without demonstrable carcinogenic activity. Failure of these procedures to yield active preparations turned our attention to the techniques employed in another system, for example, in *Drosophila*.

Extensive investigations demonstrated that acellular preparations of larval extracts of *tu-e* (spontaneous tumor) strain of *D. melanogaster* contain an inherited tumor-inducing factor.[12] The application of protein fractionation techniques to the purification of *tu-e* extracts resulted in approximately a 12,000-fold purification while maintaining a high level of activity of tumor-inducing factor.

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**This term is defined below under DISCUSSION.

This communication is concerned with: (1) the description of a purification technique (BFK); (2) the application of this technique to the refinement of active tumor factor; and (3) the induction of a variety of neoplasms in mice following the injection of refined tumor factor.

MATERIALS AND METHODS

Preparation of Extracts

TF preparations were obtained from mouse and human sources. Mouse tissues were liver, spleen, lymph nodes, thymus, and brain of AK mice with spontaneous or transplanted leukemia and of C3H(f) mice with induced leukemia. Human tissues were lymphosarcomatous lymph nodes, spleen, and lymph node from patients with HD, buffy coat from myelogenous leukemic blood, and serum or ascites fluid from patients with HD.

In the BFK procedure (TABLE 1) the purification methods of Burton *et al.*[12] were modified where differences in tissue made it necessary. The heat treatment, ammonium sulfate precipitation, and dialysis used in the original procedure of Burton *et al.* were omitted. Details of the three steps of the process are as follows:

Step 1. Preparation of crude extracts. (1) Fifteen gm. of tissue plus 150 ml. of Tris buffer (*p*H 7.8, 0.05 M) were homogenized in a Vir-Tis homogenizer for 30 sec., and the homogenate was centrifuged at 6500 × g for 15 min. in a Servall SS-1 centrifuge. The sediment was discarded, and the supernatant fluid was the crude tissue extract; and (2) clotted whole blood or ascites fluid was centrifuged at 3860 × g for 5 min. To the resultant supernatant fluid an equal volume of Tris buffer (*p*H 7.8, 0.05M) was added, and this mixture was centrifuged at 6500 × g for 15 min. The sediment was discarded, and the supernatant fluid was the crude (serum or ascites) extract. Thereafter both of these crude extracts were handled in like manner.

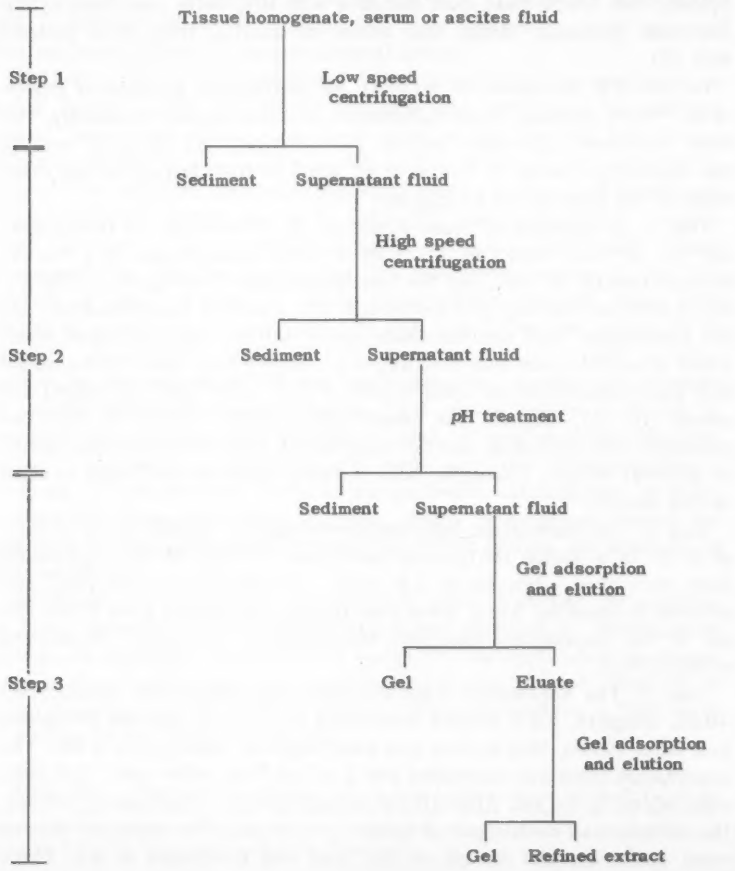
Step 2. The supernatant fluid was centrifuged at 35,000 × g for 15 min. at 0° C. in a Spinco refrigerated centrifuge. The *p*H of the supernatant fluid was then adjusted to 5.0 (with 1 M solution of KH_2PO_4) and allowed to stand for 2 min. After centrifugation at 9800 × g for 2 min. the *p*H of this supernatant fluid was readjusted to 5.0 (with 1 M solution of Na_2HPO_4).

Step 3. The supernatant fluid obtained from step 2 was divided into 40-ml. aliquots. Each aliquot was added to 3 ml. of calcium phosphate gel. After 10 min. this mixture was centrifuged at 3860 × g for 2 min. The supernatant fluid was discarded and 3 ml. of Tris buffer (*p*H 7.5, 0.05M) were added to the gel. After 10 min. of agitation in a mechanical shaker, the mixture was centrifuged at 3860 × g for 2 min. The resultant eluates were combined, and the *p*H of this pool was readjusted to 6.5. Three

milliliters of fresh calcium phosphate gel were added to this pool, and after 10 min. this mixture was centrifuged at 3860 × g for 2 min. The supernatant fluid was discarded, and 3 ml. of Tris buffer (*p*H 7.5, 0.05M) was added to the sediment. After 10 min. of agitation on a mechanical shaker the mixture was centrifuged at 3860 × g for 2 min. The supernatant fluid constituted the refined extract, and it was ampulated and stored in dry ice.

TABLE 1

BFK PURIFICATION PROCEDURE



Testing of Extracts

Refined preparations were inoculated into newborn C3H(f) hosts, either subcutaneously or intravenously.[7] The C3H(f) strain of mice used as bio-assay animals in this investigation has been inbred in our laboratory for 3½ years. Only one spontaneous leukemia has been found in several thousands of our hosts, and we have not observed any other spontaneous neoplasms in this line. Since this C3H(f) line, unlike AK-C3H(f) hybrids, apparently has a high resistance to spontaneous neoplasia, it was selected as the source of host animals.

Control animals were inoculated with Tris buffer. Moreover, a procedural control was used. As used routinely, in the control of both chemical and immunological procedures, a control was included to ensure that neither physical procedures nor chemicals produced the observed end result. It had been observed in the refinement of *Drosophila* tumor-inducing factor that the omission of high-speed centrifugation from the procedure always yielded preparations without demonstrable tumor-inducing activity (unpublished data). Refined mammalian TF was prepared, as per the aberrant *Drosophila* technique (omission of high-speed centrifugation), and bio-assayed in C3H(f) hosts. As a further control, refined extracts prepared from serum of "normal" human adults were inoculated into C3H(f) hosts.

RESULTS

The results are summarized in TABLE 1. TF obtained from mouse leukemic tissues induced tumor formation in 60 of 85 hosts, whereas TF acquired from human neoplastic sources induced tumor formation in 29 of 228 hosts. In individual mouse experiments, tumor induction ranged from 60 to 100 per cent whereas, in those experiments using TF from human sources, tumor induction was from 4 to 30 per cent. All tumors were verified by biopsy and/or on post-mortem examination.

Tumors induced in the C3H(f) hosts by TF extracted from mouse neoplastic material were identified as parotid, mammary and adrenal carcinomas, fibro- and osteogenic sarcomas, and one myxoma. Neoplasms induced in host animals by TF extracted from human sources were identified as parotid and mammary carcinomas, fibrosarcoma, and one leukemia (this leukemia has been successfully transplanted into young adult C3H(f) hosts; the inoculation of these cell suspensions resulted in 100 per cent takes). Multiple tumors occurred frequently and often involved the simultaneous presence of more than one tumor type.

All of the induced neoplasms were identified by histologic examination and were comparable to previous descriptions of such tumors.[1,4,5] Histologic studies of individual induced tumor types did not reveal any recognizable differences between tumors induced by TF from mouse or human sources.

None of the 455 hosts injected with control preparations (TABLE 2) developed neoplasms (200 to 300 days postinoculation). This is a clear indication that neither Tris buffer, the chemicals, nor the physical procedures conferred carcinogenic activity upon the refined TF. It must be emphasized that, although the tissue source contained active TF and the purification procedure used was identical except for the omission of high-speed centrifugation, the resultant preparations were inactive. The controls further indicate that the refined preparations of serum from "normal healthy" human adults did not contain active TF.

DISCUSSION

Although Hodgkin's disease was not induced in the mouse, the results indicate that the BFK procedure may be successfully applied to the isolation and refinement of active TF from HD tissues, sera and ascites fluid; buffy coat from myelogenous leukemic blood; and leukemic mouse tissue. Refined TFs obtained from varied sources induced the same variety of neoplasms in our C3H(f) hosts. In contrast, there have been no reports* of crude preparations of human material inducing the formation of solid tumors in the mouse. Such crude preparations, although containing TF, also possess a multitude of biologically active substances, that is, enzyme systems, micro- and submicrocellular particulates, and by-products of cell breakdown, which may mask, inactivate, or destroy the tumor inductive potential in crude preparations. The elimination of these substances by the BFK procedure is reflected by the reproducibility of TF isolation, the greater control over experimental procedures, and the enhancement of TF activity. Although TF activity may be enhanced by other methods, for example, tissue culture[14,15] and *in vivo* passage, these methods may not eliminate biologically active substances.

The presence of the modifying substances may have been responsible, in part, for the former lack of tumor-inducing activity of preparations of human neoplastic tissues inoculated into mice. Thus, refinement by the BFK procedure removed substances responsible for the maintenance of the species-specificity barrier. It is proposed that tumor factor, although isolated from two divergent Metazoan sources, may participate in a common tumor-inducing mechanism. This common mechanism is suggested by the observation that TF isolated from two neoplastic sources, mouse or human, by the same technique induced histologically indistinguishable tumors in our C3H(f) hosts.

The BFK procedure is a stepwise use of protein fractionation techniques and was developed by the modification of techniques used for the extraction and refinement of tumor-inducing factor present in the *tu-e* strain of *D. melanogaster*. These techniques removed extraneous

*A recent report mentions the development of two parotid tumors in mice inoculated with extracts of human leukemia.[13]

TABLE 2

	Number of exper.	Source of tissue	Dose and route of administration	Survived to weaning of no. inject.	No. tumors and no. tumor animals	Time of tumor detection	Tumor type and number
BFK Extracts of mouse leukemic tissue	4	L. Nodes Spleen Liver Thymus Brain	0.01 to 0.1 ml. SC 0.01 to 0.05 ml. IV	85/155	108/60	70 to 180 days	88 Parotid 11 Mammary 1 Adrenal 6 Fibrosa. 1 Myxoma 1 Osteosa.
BFK Extracts of human tissue	8	Buffy coat (Myel. L) Spleen, HD Node, HD Blood, HD Asc. Fl. (HD)	0.03 to 0.15 ml. SC 0.02 to 0.05 ml. IV	228*/420	43/29	93 to 240 days	39 Parotid 2 Mammary 1 Leukemia 1 Fibrosa.
Tris 0.05 M *p*H 7.5	2		0.05 ml. IV 0.1 ml. SC	43/60	0	Sacrificed at 210 days	0
BFK Prep. high-speed centrifugation omitted	8	Mouse	0.05 ml. IV 0.1 ml. SC	155/333	0	Sacrificed at 210 days	0
	8	Human	0.03 to 0.05 ml. IV 0.1 ml. SC	257/348	0	Sacrificed at 200 to 300 days	0

* 131 animals still living.

substances while maintaining TF activity. The successful application of the BFK procedure to mouse and human neoplastic sources confirms the postulate by Kassel[16] that active TF could be isolated from mammalian neoplasms.

The control experiments (TABLE 1) clearly indicate that the diluting medium is not carcinogenic. Furthermore, the control experiments indicate that the sequence of procedures in the BFK technique must be strictly adhered to if the activity of TF is to be maintained. The omission of high-speed centrifugation, as observed during the refinement of *Drosophila* tumor-inducing factor, resulted in the production of preparations with no demonstrable tumor-inducing activity. It has been established that submicroscopic particulates are sedimented by high-speed centrifugation and that some of these particulates are associated with cellular enzyme activity.[17-20] It is possible that *p*H alteration may stimulate these enzymes to inactivate the TF. Thus, the removal of submicroscopic particulates by high-speed centrifugation prior to *p*H treatment is critical to the maintenance of TF activity.

The use of the term tumor factor (TF) in this paper refers to the substance or substances responsible for tumor induction. The substance(s) has been extracted and refined from both mouse and human neoplastic sources. The tumor-inducing entity was not called "tumor virus" because its biological and chemical identity has not been established. The identification of this entity was deemed of less immediate importance than the elucidation of its role in carcinogenesis. In addition, the premature labeling of the entity could prejudice the direction of investigations.

The part played by TF in oncogenesis is enigmatic. However, since spontaneous neoplasia is extremely rare in our hosts and total tumor induction was significant following injection of refined preparations, TF may be a prime participant in the observed carcinogenesis.

It may be suggested by some investigators that the induction of tumor is mediated through the activation of a "latent tumor virus" present in our hosts. Since the presence or absence of an inactive "tumor virus" has not been demonstrated conclusively by available methods, its existence must remain questionable. However, even if the presence of a "latent tumor virus" was established in our C3H(f) hosts, the significance of TF, refined from human and mouse neoplasia, would not be that of a common triggering mechanism.

SUMMARY

Refined extracts of human and mouse tumor tissue have been prepared by a technique called the BFK technique.

Refined tumor factor has been extracted from both human and mouse tumor tissue. The injection of the refined extracts into C3H(f) mice

induced primary parotid tumors, mammary tumors, adrenal tumors, soft tissue tumors, and leukemia.

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DIVISION OF MYCOLOGY

COMMENTS ON YEASTS SEEN IN THE MEDICAL MYCOLOGY LABORATORY*

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There are few diagnostic medical bacteriology laboratories that are not isolating yeasts from clinical materials. Unfortunately, many of these laboratories do not have personnel trained in medical mycology; thus, little attempt is made to classify any of these organisms with the exception of the *Candida* genus. In many instances methods for recognizing *C. albicans* alone are employed. This is unfortunate, as other species of *Candida* may also be pathogenic, particularly *C. tropicalis* and *C. krusei*. Experiences in our own laboratory during recent years have impressed this fact upon us. Although most instances of thrush, cutaneous candidiasis are usually due to *C. albicans*, we have also observed *C. tropicalis* and *C. krusei* as the etiological agents for these infections. Many physicians do not realize that these species may also be pathogenic and, thus, consider any report positive for *Candida* species other then *C. albicans* as insignificant. Although we have isolated *C. parakrusei* from clinical materials, it has not been observed as the causal agent for any form of candidiasis. On the other hand, we have recently seen a patient with cutaneous candidiasis due to *C. guilliermondi*.

A clinical form of candidiasis not yet recognized by many physicians and mycologists is referred to by B. M. Harvard of the Yale-New Haven Medical Center, as "familial venereal candidiasis." Harvard and his associates believe that a *Candida* infection of the prepuce and glans penis in uncircumcized males may be considered as a form of venereal disease, the infection being continually perpetuated by either of the marital partners. This particular type of *Candida* infection is very characteristic clinically.

The first symptom presented by the patient is a pruritus of the preputial meatus; examination will reveal a mild irritation around the glans and the mucosal surface of the prepuce; frequently a slight seropurulent discharge will be observed. In more severe instances there may be mild swelling of the glans, intensifying the itching and burning sensation and causing much discomfort to the patient. The mucosa

*This paper, illustrated with slides, was presented at a meeting of the Division on May 22, 1959. Some of the comments included in this article are based upon studies supported by Grant E-1641, National Institute of Allergy and Infectious Diseases, Public Health Service, Bethesda, Md.

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around the glans will appear moist and there is usually a whitish secretion. On the surface of the glans there may be seen erythematous macules approximately 1 mm. in diameter. Microscopic examination of material from one of these lesions will reveal the typical pseudohyphae of *Candida*, and cultures will be positive for *C. albicans*. The patient, when questioned, will usually indicate a history of a vaginal discharge in his wife.

At times it is not possible to obtain material for mycological studies but, because of the typical clinical picture and history, treatment for candidiasis is given with excellent results. This treatment consists of anointing the pubis, testes, and entire penis with propionate jelly before retiring; the next morning the area is thoroughly washed. Care is taken to cleanse and dry the preputial cavity. To ensure a cure and reduce the chance of reinfection it is recommended that the wife be seen by a gynecologist for treatment of the vaginal discharge. Circumcision is recommended for the husband. For best results treatment is carried out simultaneously in both partners.

The significance placed upon cultures positive for *C. albicans* or other species is often erroneous. *Candida* species may be found as normal flora of any area of the body, including the skin. Thus, a positive culture for *Candida* cannot be interpreted properly unless microscopic studies of the original material are carefully done. *Candida* may be considered to be dimorphic; it produces pseudohyphae, even true hyphae, as well as blastospores when it is invading tissue. Only budding forms are seen when the organism is saprophytic. Indeed, the laboratory confirmation of a diagnosis of thrush, cutaneous candidiasis, and even vaginitis due to a *Candida* infection may be made on the microscopic examination of material properly collected from lesions present in such infections. With other materials such as sputum it is also important to demonstrate the hyphal elements in a fresh specimen before cultures positive for *Candida* can be considered significant. It may require examination of several specimens before one finds the pseudohyphae, despite the fact that cultures may be consistently positive for the organism.

The actual identification of *C. albicans* poses a greater problem to the laboratory than many mycologists realize. We have attempted to re-evaluate the current techniques as well as media used in the study of these organisms in order to facilitate recognition of this group by laboratories that do not have mycological consultants. These studies have been published elsewhere,[1,2] but will be summarized here.

It is essential that yeasts examined by these techniques be grown for two generations on a sugar-free medium; if this step is omitted, the number of strains of *C. albicans* requiring fermentation studies for identification will rise significantly because of failure to develop typical spores on a chlamydospore medium.

The amount of inoculum used with the chlamydospore medium is very important. If a heavy inoculum is used, the *Candida* species will fail to produce any pseudohyphae, much less chlamydospores; in such instances the organism is usually reported out as "not a *Candida*" and discarded. We have found that less than 1 per cent of our isolates of *C. albicans* require fermentation studies for identification when we follow these precautions.

There are several media on the market that are as satisfactory as the classic corn meal agar;[3] these include zein, rice extract, and chlamydospore agars. Until recently it was not possible to obtain the zein and rice media commercially; it was therefore necessary to make them from the "raw" materials. Recent studies employing the "home-made" zein[4] and rice extract agars[5] in conjunction with the commercial forms of these media have shown that approximately 79 per cent of our strains of *C. albicans* developed chlamydospores on the commercial zein in contrast to 94 per cent producing these spores on the home-made zein. All strains of *C. albicans* used in this study produced chlamydospores on the commercial rice-extract medium in contrast to 97 per cent of the strains producing the typical spores on the home-made rice agar.

We are using the Bi, GGY, and ABY media* for the primary isolation of *C. tropicalis* and *C. albicans* from sputum. Only three strains of *C. albicans* have failed to develop their characteristic growth on these media. We are using these media in conjunction with our routine procedures, but are of the opinion that eventually they may reduce the instances in which one must employ chlamydospore agar.

During the past 18 months we have observed a yeast that we believe must be considered as much a part of the normal flora of the skin and the intestinal and respiratory tracts as are the *Candida* species. This organism is *Torulopsis glabrata*. It was first recognized by our laboratory during a study of quantitative methods for bacterial cultures on urine specimens.[6] During this work we observed 15 patients who had yeasts present in numbers varing from 10,000 to 37,000,000 organisms/ml. of urine. Nine of these patients had diabetes mellitus, and 12 had received antibiotics during the preceding year; 9 of these 12 patients acquired the fungus during the course of treatment for a bacterial infection of the urinary tract. The yeast isolated from 6 of these patients was *C. albicans;* 7 patients had *Torulopsis glabrata,* and 2 patients had an unidentified species of *Torulopsis*. Four of the strains of *T. glabrata* were sent to Lynferd Wickerham with the request that he confirm their identity.

Wickerham[7] has stated that in an 18-year period he has seen only 3 strains of this fungus, but that since March 1956 he has received 6 strains for identification, all isolated from patients: 2 with septicemia.

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He thought the attention of physicians and technologists should be called to this organism because, in some instances, it might cause serious infections.

We have continued to isolate *T. glabrata* from urine specimens of patients with varied diseases. In most instances the specimens have contained 10,000 organisms or more/ml. of urine. Since only 1 specimen was received from most of these patients, it is not possible to interpret the significance of these observations. Obviously, more intensive studies must be undertaken with regard to this problem. Certainly, the report of Guze and Haley[6] indicates that the patients included in their study had a true infection with this yeast.

T. glabrata has been recovered from stools, sputum, and skin of normal individuals. It has also been recovered by us from the eye and the blood stream, but in these instances there was no evidence that the organism was anything more than a contaminant.

Although our studies with *T. glabrata* are still in progress, we believe that this organism is not highly pathogenic, but that it may, under as-yet-undefined conditions, cause a serious infection.

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*DIVISION OF ANTHROPOLOGY**

EARLY MAN IN CAVE AND VILLAGE AT SHANIDAR, KURDISTAN IRAQ†

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The Smithsonian Institution-sponsored archaeological expedition to Shanidar Valley in northern Iraq made several important discoveries during its 1956-1957 season.‡ These new finds are the remains of three adult Neanderthals and the location of a proto-Neolithic open habitation site. No less important was the recovery of data that will give us an understanding of the kinds of plants and animals that flourished there in the past, as well as an idea of their contemporary climates.

The skeletal finds at Shanidar Cave provide the students of paleoanthropology with new data, information badly needed in the study of Early Man in the Near East. For the first time, we are in a position to gauge the age of Neanderthals by means of carbon-14 dating. Toward the other end of the time scale, the discovery of the early habitation site, Zawi Chemi Shanidar, of proto-Neolithic age, was most fortunate. This site, situated close to Shanidar Cave, ties in culturally with the upper part of the cave stratigraphy. The chronological contemporaneity of the two is verified by matching carbon-14 dates.

Shanidar is a small Kurdish village and police post in the Zagros Mountains about 250 airline miles north of Baghdad. It lies on the Greater Zab River, a major tributary of the Tigris River. The site of Zawi Chemi Shanidar lies in a small cultivated field just upstream from Shanidar village, on the first terrace above the river. Shanidar Cave is approximately 2½ miles away, more than one thousand feet up Baradost Mountain, whose precipitous heights tower above the Shanidar valley to the northeast. When the proposed Bekhme Dam, about 8 miles below Shanidar village is completed, the habitation site and village will both be inun-

*The Division of Oceanography and Meteorology held a meeting on May 26, 1959 at which Bernhard Haurwitz of the Department of Meteorology and Oceanography, New York University, New York, N. Y., presented a paper entitled "Survey of the General Circulation of the Atmosphere." This paper will not be published by the Academy.

†This paper, illustrated with slides, was presented at a meeting of the Division on May 25, 1959.

‡The expedition was supported by grants from several organizations, including the American Philosophical Society, Philadelphia, Pa., the William Bayard Cutting Travelling Fellowship of Columbia University, New York, N.Y., the National Science Foundation, Washington, D.C., the Bruce Hughes Fund of the Smithsonian Institution, and the Wenner-Gren Foundation for Anthropological Research, New York, N.Y. The Iraq Petroleum Company, Limited, graciously lent material aid and assistance to the expedition in Iraq. The Directorate General of Antiquities of Iraq, as in the 1951 and 1953 seasons (Solecki, 1955), extended its cooperation. The field personnel of the Shanidar 1956-1957 expedition included the author; his wife, Rose Lilien Solecki, Columbia University; Philip Smith, Peabody Museum, Harvard University, Cambridge, Mass.; and George Maranjian, of the Arabian-American Oil Company at Dhahran, Saudi Arabia.

dated. Shanidar Cave will be spared, since it lies above the flood-pool elevation of the proposed reservoir. However, it will be extremely difficult to excavate.

The cave was excavated during the fall and spring season of 1956-1957. The open habitation site was tested during the fall season and more fully explored during the spring season. The major work of the expedition was at Shanidar Cave. This is an immense cavern that even today is the winter home of several families of Kurdish herders. The excavation of former seasons was enlarged to form a cut measuring 20 meters long by 7.75 meters wide at the top. It was stepped back in depth to the bottom, or bedrock, at a depth of nearly 14 meters.

The four major cultural layers outlined previously at Shanidar Cave were investigated further. These layers from top to bottom were identified as Recent to Neolithic (Layer A); Mesolithic (Layer B); Upper Paleolithic, or Baradostian (Layer C); and Middle Paleolithic, Mousterian (Layer D). The industries from the Mesolithic and the Mousterian layers appear to be similar to the industries from Zarzi Cave and Hazer Merd Cave, respectively, sites also in Kurdistan Iraq. The latter sites were explored by Dorothy Garrod (1930), formerly of Cambridge University, Cambridge, England, in 1928. The Baradostian is a name I have given to the blade and burin industry in Layer C. It is like the Aurignacian of the West.

Thus far, we have 13 carbon-14 dates from Shanidar Cave, mainly from the Baradostian layer. The dates, as yielded by 4 different laboratories in this country and abroad, correlate very well, with a small percentage of error on duplicate samples. One of the objectives of the 1956-1957 season, in which the carbon-14 dating played a part, was to close the cultural hiatus between the Mesolithic and the Baradostian layer, and the Baradostian and the Mousterian layers. It was confirmed that the cultural and chronological gaps are real, at least in the limits of the present excavation. The former hiatus falls approximately between 12,000 and 27,000 years ago. This is about the time of the Würm Maximum in the Alpine sequence of glaciation. The second hiatus is delimited with somewhat less certainty. The base of the Baradostian appears to be close to 34,000 years in age from a series of 4 carbon-14 dates. H. de Vries of Rijks University, Groningen, the Netherlands, has dated a sample from the top of Layer D as 50,000 ± 3000 to 4000 years of age, adding the qualification that there is a 10 per cent possibility that the sample is older than 60,000 years (letter of May 25, 1959). This hiatus accordingly may be at least 15,000 years.

Regarding studies of other nonartifactual remains, Charles Reed of the University of Illinois, Urbana, Ill., who is making an examination of the mammal remains from Shanidar, has an interesting observation. He noted from the faunal remains that no great environmental change is reflected at Shanidar Cave from its first occupation about 90,000 to 100,000 years ago (my estimation) to the present. This opinion seems to be confirmed by the

types of snail shells found in the deposits. Analysis of the soil for fossil pollen, which is now being done with some encouraging results, will give us a check on Reed's observation. The cultural remains together with the nonartifactual evidence framed against the chronological sequence will give us an excellent picture of prehistoric life and its times at Shanidar Cave.

During the three seasons, the unmineralized skeletal remains of 7 individuals were found in Shanidar Cave. Three skeletons were found in the Neolithic to Recent layer, and 4 were found in the Mousterian layer. The latter include the three Neanderthal remains, which were called Shanidar I, II, and III, and the remains of the Shanidar infant, not numbered. The infant remains, discovered in 1953, were studied by Muzaffer Senyürek (1957), of Ankara University, Ankara, Turkey, during the winter of 1956 in Baghdad.

Shanidar I, the most complete adult skeleton of the group, was found at a depth of 4.3 meters near the top of the Mousterian layer in the east wall of the excavation. Shanidar II was found at a depth of 7.3 meters in the west wall of the excavation. Shanidar III was found close to Shanidar I at a depth of 5.4 meters. These individuals, identified as Neanderthals, had evidently been crushed to death by falls of rocks from the cave ceiling.

The skeletal remains of Shanidar I were found extended at full length, twisted and broken under a heap of stones. From his position, he must have been on his feet when he was killed. Several bones of his lower limbs were out of place, possibly disturbed by animals. A low heap of portable-sized stones was found directly over the remains. This would indicate that the survivors had returned and thoughtfully covered him with additional stones. A number of mammal bones was found among these stones, particularly over the center. It does not seem probable that these bones were amassed there by animals. Many of the bones were tightly wedged, and some were even crushed under the stones. The alternate solution is that the builders of the cairn had themselves left these bone remnants, presumably part of a meal. Whether this was part of a ceremony is conjectural. However, Neanderthal remains have been found ritually buried elsewhere.

I have originally estimated an age of about 45,000 years for Shanidar I (Solecki, 1957), a somewhat modest figure in the light of de Vries's recent findings. De Vries's unpublished date of 50,000 years for the top of the Mousterian layer, although taken from a carbon sample found in another part of the excavation, I consider to approximate the age of Shanidar I (barring any accident of stratigraphy). Bearing in mind de Vries' qualification about this date, we may now say with some confidence that we have an absolute date for a Neanderthal.

Shanidar III, represented by some articulated vertebrae, ribs, and other parts of the lower torso, smashed lower limbs and some other loose bone fragments, was found less than 3 meters away from Shanidar I. It was sep-

arated by a thin mantle of rocks from the latter skeleton. There is at most a difference of a few hundred years, Shanidar III being the older. This individual had been unquestionably caught in a rockfall. His upper extremities had been sheared away. Bone meal and splinters were found in the churned mass of stones and yellow loam, mute evidence of the force of the ancient catastrophe.

Shanidar II, represented by a laterally flattened skull, was found under some stones next to a thick hearth in the west wall of the excavation. The skull faced to the east, the lower jaw open and broken over a stone. From the position of this find, it is probably several thousand years older than either Shanidar I or III, perhaps as much as 60,000 years old.

The rockfalls that had killed the adults were presumably dislodged by earthquakes, which occur frequently in the Zagros Mountains. The crushed and broken remains are testimony to the fact that all of the dangers during the stone age were not outside the cave, and that the cave inhabitants were not safe even in the protection of their own homes.

On my suggestion to the Directorate General of Antiquities of Iraq, T. Dale Stewart (1958, 1959) of the United States National Museum, Smithsonian Institution, was invited to Baghdad to study the Shanidar Neanderthals. Stewart accepted the invitation and went to Baghdad in the fall of 1957. He was impressed with the primitive characteristics of the Shanidar I skull which he restored. Using measurement scales obtained from modern bones, Stewart found that the skeletal remains of Shanidar I suggest an individual about 5 feet 7 to 8 in. tall. Shanidar I is believed to be a male who was about 40 years old at death. A puzzle that confronted us in the field was resolved when Stewart found that Shanidar I was a cripple, with a useless stump of a right arm. This arm, which had never fully developed, may have been amputated at the elbow. Unusual heavy attritional wear on his incisor and canine teeth suggests that the front teeth were employed in lieu of an arm. This particular cave man must have had some special status, crippled though he was, and probably earned his subsistence.

The nearest locus of ancient human skeletal finds in significant numbers to which the Shanidar skeletons may be compared is Mt. Carmel in Palestine. However, the situation there is not straightforward and simple. Neanderthal skeletons of both primitive or "conservative" and more humanly advanced or "progressive" types were recovered in two different caves. These are purported to date from the Riss-Würm interglacial period equivalent in the Near East, according to the finders. Others believe that the skeletons belong to a later period. One investigator, F. Clark Howell (1958) of the University of Chicago, is of the opinion that the Mt. Carmel remains are of the Last Pluvial period (equivalent to Würm I). This estimate is closer to our own dating for the Shanidar finds, which are Würm 1-2 interstadial in age in the Alpine sequence of glaciation. There is still the problem of the "conservative" and "progressive" Neanderthal types at Mt. Carmel. The former are represented by the Tabun skeletal remains,

the latter by the Skhul skeletons. The Shanidar Neanderthals I, II, and III match more closely the conservative Tabun rather than the anomolous progressive Skhul Neanderthals. The latter may represent a variation in a single population, a new set of peoples on the contemporary scene, or a later group with characteristics of modern man. Detailed comparisons of the artifactual and nonartifactual assemblages between Shanidar Cave and the caves of Mt. Carmel should provide suggestions to the solution of the long-standing problems at Mt. Carmel. At any rate, the view that the Mt. Carmel remains date from the Riss-Würm interglacial is implausible. We cannot believe that the Shanidar remains represent a population of Neanderthals who languished in the Kurdish mountains many thousands of years after their physical counterparts (not to speak of the Skhul Neanderthals with modern man characteristics) disappeared in Palestine.

The early habitation site of Zawi Chemi Shanidar is in an entirely different realm of prehistory. It represents a whole new set of problems in a field of investigation now receiving concentrated interest in Iraq. One of our two initial test trenches made at Zawi Chemi Shanidar fortunately exposed the habitation remains. It was found at the bottom of a 1.5-meter-deep cut in Layer B, the basal layer of the site. Judged by the ceramics, Layer A, the top layer, appeared to date from between the Sixth and Twelfth Centuries A.D. Layer B yielded a preceramic inventory of artifacts similar to the material from Karim Shahir and related sites excavated in Iraq by the University of Chicago Oriental Institute expeditions (Braidwood, 1958). Associated with the layer is an ovoid structure of rough field stones measuring about 3 meters in diameter. It resembles a primitive sort of architectural foundation (Solecki and Rubin, 1958). A carbon-14 sample was dated 10,870 years ± 300 years old from this site. This date closely matched one for the upper part of Shanidar Cave, where similar artifacts were found.

Unfortunately, no botanical remains evidencing agriculture were discovered. However, a large number of trough querns and mullers or rubbing stones, probably used for grinding cereal grains, were found. No sickle blades were recovered, but a large number of quartzite spalls in the assemblage possibly served as handy cutting implements. There was an abundance of goat or sheep bones. It is possible that these animals were already tended over ten thousand years ago in Kurdistan. The whole aspect of Zawi Chemi Shanidar is one of primitiveness. It is very likely that this site was seasonally occupied, the people taking winter refuge in Shanidar Cave and other neighboring shelters. This custom is followed in the Shanidar area today.

In describing the present status of information about Shanidar Cave and the habitation site of Zawi Chemi Shanidar, it must be remembered that research at both of these sites is still continuing. As the data are sifted and analyzed, new information is accumulated, adding to or modifying the

interpretations already made. The Shanidar finds have added material knowledge not only to the prehistory of Iraq, but also to the Near East. Shanidar is unique in that here we have a laboratory evidencing the development of man in one place, from early stone age to neolithic times.

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CONFERENCES HELD

SECTION OF BIOLOGICAL AND MEDICAL SCIENCES

RECENT CONTRIBUTIONS TO ANTIBACTERIAL THERAPY

Thursday, May 21, and
Friday, May 22, 1959

Conference Chairman: Louis Weinstein
Pratt Diagnostic Clinic and New England Medical Center, Boston, Mass.

THURSDAY, MAY 21, 1959

Session Chairman: Paul S. Rhoads
Northwestern University School of Medicine, Chicago, Ill.

9:00 A.M.-
Greetings from the Academy - Robert L. Kroc, Chairman, Section of Biological and Medical Sciences, The New York Academy of Sciences, New York, N. Y.; Warner-Lambert Research Institute, Morris Plains, N. J.

"Pharmacological Studies with Sulfadimethoxine" - R. E. Bagdon and L. O. Randall, Department of Pharmacology, Hoffmann-La Roche, Inc., Nutley, N. J.; and W. A. Leff, St. Barnabas Medical Center, Newark, N. J.

"Comparative Chemotherapeutic Studies with Modern Sulfonamides" - W. F. DeLorenzo and R. J. Schnitzer, Department of Chemotherapy, Hoffmann-La Roche, Inc., Nutley, N. J.

"The New Low-Dosage Sulfonamides Are Different" - W. P. Boger, Norristown State Hospital, Norristown, Pa.; and B. Koechlin, Biochemical Department, Hoffmann-La Roche, Inc., Nutley, N. J.

"Comparative Sensitivity of Pathogenic Bacteria to Modern Antibacterial Agents" - B. H. Leming and Clyde Flanigan, Division of Pathology and Microbiology, University of Tennessee and City of Memphis Hospital, Memphis, Tenn.

"Treatment of Complications of the Upper Respiratory Tract" -

(1) T. D. Michael, University of Maryland School of Medicine, Baltimore, Md.
(2) S. M. Finegold, Veterans Administration Center, Los Angeles, Calif.
(3) William J. Grace, St. Vincent's Hospital, New York, N. Y.

Discussant: J. P. Sanford, Southwestern Medical School of The University of Texas, Dallas, Texas.

Session Chairman: A. J. Vignec
Pediatric Division, St. Vincent's Hospital and
Clinical Professor of Pediatrics,
New York University-Bellevue Hospital, New York, N. Y.

2:00 P.M.-

"Sulfadimethoxine in Lingual Tonsillitis" - J. C. Elia, Washoe Medical Center and St. Mary's Hospital, Reno, Nev.

"Low Dosage Sulfonamides in the Treatment of Respiratory Infections" - L. E. Skinner, Lakewood Clinic, Tacoma, Wash.

Discussant: G. A. Moore, Worcester State Hospital, Worcester, Mass.

"Modern Sulfonamides in Pediatric Practice" -

(1) C. W. Daeschner, Baylor University College of Medicine, Houston, Texas.

(2) E. H. Townsend, Rochester General Hospital, Rochester, N. Y.

Discussant: Saul Krugman, New York University College of Medicine, Bellevue Medical Center, New York, N. Y.

"Chemotherapeutic Treatment of Acne" -

(1) S. W. Levy, University of California, School of Medicine, San Francisco, Calif.

(2) M. M. Cahn and E. J. Levy, University of Pennsylvania Medical School, Philadelphia, Pa.

"Low Dosage Sulfonamides in Urology" -

(1) J. W. Draper and M. Serpe, Cornell University Division, Department of Urology, Bellevue Hospital, New York, N. Y.

(2) J. D. Young, Jr., S. Kiser, and D. O. Beyer, University of Maryland School of Medicine, Baltimore, Md.

Discussant: Grayson Carroll, St. Louis University School of Medicine, St. Louis, Mo.

FRIDAY, MAY 22, 1959

Session Chairman: C. S. Livingood
Henry Ford Hospital, Detroit, Mich.

9:00 A.M. -

"Experimental Approach to Topical Antibacterial Therapy" - E. Grunberg, Department of Chemotherapy, Hoffmann-La Roche, Inc., Nutley, N. J.

"Triclobisonium Chloride, Neomycin, Bacitracin and Other Antibacterials in a Variety of Dermatological Conditions" -

(1) S. Bluefarb, Department of Dermatology, Northwestern University School of Medicine and Cook County Hospital, Chicago, Ill.

(2) E. Edelson, Department of Dermatology, Newark Department of Health and Welfare, Newark, N. J.
(3) F. T. Becker, The Duluth Clinic, Duluth, Minn.
(4) P. L. Williams, Doctors Hospital, Seattle, Wash.

"The Use of Triclobisonium Chloride in Pediatric Practice" - A. B. Falk and J. M. Fox, Children's Memorial Hospital and Illinois Research Hospital, Chicago, Ill.

Discussant: R. C. V. Robinson, University of Maryland Medical School Baltimore, Md.

"Topical Therapy in Acne Vulgaris" - S. L. Hanfling, Orange Memorial Hospital, Orange, N. J.

Discussant: S. W. Levy, University of California School of Medicine, San Francisco, Calif.

Session Chairman: Robert Turell
Mt. Sinai Hospital and Albert Einstein College of Medicine,
New York, N. Y.

2:00 P.M. -
"The Use of Topical Antibacterials in Surgical Procedures" -

(1) *General Surgery* - R. D. Floyd and W. Anlyan, Duke University Hospital, Durham, N. C.

(2) *Plastic Surgery* - N. Georgiade, Duke University Hospital, Durham, N. C.

(3) *Proctology* - G. L. Becker, Paterson General Hospital, Paterson, N. J.

"The Control of Infections in Burn Therapy" - Jerome Gelb, Kessler Institute for Rehabilitation, West Orange, N. J.

Discussant: D. M. Mayer, New York Medical College, Flower and Fifth Avenue Hospitals, New York, N. Y.

"Local Therapy in Vaginitis" -
(1) N. Mulla, St. Elizabeth's Hospital, Youngstown, Ohio
(2) L. E. Savel, Newark Beth Israel Hospital, Newark, N. J.

Discussant: B. J. Pisani, St. Vincent's Hospital, New York, N. Y.

Robert L. Kroc
Chairman

Charles Noback
Secretary

DIVISION OF ANTHROPOLOGY

SOCIAL AND CULTURAL PLURALISM IN THE CARIBBEAN

Wednesday, May 27, and
Thursday, May 28, 1959

Conference Co-Chairmen
Vera Rubin, Research Institute for the Study of Man, New York, N. Y.
Dorothy L. Keur, Hunter College, New York, N. Y.

WEDNESDAY, MAY 27, 1959

Session Chairman: Manning Nash
University of Chicago, Chicago, Ill.

9:00 A.M.-
Greetings from the Academy - Dorothy L. Keur, Chairman, Division of Anthropology, The New York Academy of Sciences, New York, N. Y.; Hunter College, New York, N. Y.

"Social and Cultural Pluralism" - Michael G. Smith, Nigerian Institute of Social and Economic Research, Kano, Nigeria.

Discussants: Charles Wagley, Columbia University, New York, N. Y.; Vera Rubin, Research Institute for the Study of Man, New York, N. Y.

"The Range and Variation of Caribbean Societies" - David Lowenthal, American Geographical Society, New York, N. Y.

"Metropolitan Influences in the Caribbean"
Netherlands Antilles--Dorothy L. Keur, Hunter College, New York, N. Y.
French Antilles--Michael Horowitz, Columbia University, New York, N. Y.
The West Indies--Lambros Comitas, Columbia University, New York, N. Y.

Session Chairman: Charles Wagley
Columbia University, New York, N. Y.

2:00 P.M.-
"Social Stratification and Pluralism" - Lloyd Braithwaite, Institute of Social and Economic Research, Mona, Jamaica, The West Indies.

Discussant: Simon Rottenberg, University of Chicago, Chicago, Ill.

"Peasants, Plantations, and Pluralism" - Elena Padilla, Community Mental Health Board, New York, N. Y.

Discussant: Ida Greaves, University of Pennsylvania, Philadelphia, Pa.

"Role of the Intellectual in Haitian Plural Society" - Remy Bastien, Program for Advanced Training in Social Sciences, Mexico, D. F., Mexico.

"Cultural Assimilation in a Multiracial Society" - Daniel Crowley, University of Notre Dame, South Bend, Ind.

"East and West Indian: Cultural Complexity in Trinidad" - Morton Klass, Columbia University, New York, N. Y.

THURSDAY, MAY 28, 1959

WORKSHOPS

9:30 A.M.-

I. Politics, Power, and the Plural Society

Chairman: Hugh Smythe, Brooklyn College, Brooklyn, N. Y.

"Attitudes of the Jamaican Elite Toward the West Indies Federation" - Wendell Bell, University of California, Los Angeles, Calif.

II. Urbanization, Communication, and the Plural Society

Chairman: Robert Manners, Brandeis University, Waltham, Mass.

"Urbanization and Pluralism" - Leonard Broom, University of California, Los Angeles, Calif.

WORKSHOPS

2:00 P.M.-

III. The Intellectual and the Plural Society

Chairman: Remy Bastien, Program for Advanced Training in Social Sciences, Mexico, D. F., Mexico.

"The Writer in The West Indies" - Wilfred Carty, Columbia University, New York, N. Y.

IV. Multiracialism and the Plural Society

Chairman: Michael G. Smith, Nigerian Institute of Social and Economic Research, Kano, Nigeria.

"Racial Groups and Social Class in British Guiana" - Elliott Skinner, Columbia University, New York, N. Y.

Dorothy L. Keur
Chairman

Dorothy Cross Jensen
Secretary

SECTION OF CHEMICAL SCIENCES

MOLECULAR ORGANIZATION IN SOLID HIGH POLYMERS

SATURDAY, JUNE 20, 1959

Conference Chairman: F. R. Eirich
Polytechnic Institute of Brooklyn, Brooklyn, N. Y.

Session Chairman: W. O. Baker
Bell Telephone Research Laboratories, Murray Hill, N. J.

9:00 A.M.-
Greetings from the Academy - Karl A. Folkers, Section of Chemical Sciences, The New York Academy of Sciences, New York, N. Y.; Merck and Company, Inc., Rahway, N. J.

"Problems of High Polymer Crystallinity" - H. A. Stuart, University of Mainz, Mainz, Germany.

"Growth and Organization of Polymer Spherulites" - F. P. Price, General Electric Research Laboratory, Schenectady, N. Y.

"Directional 'Crystallization' of Polymers, Examples of the Para-Crystalline State" - W. O. Statton, Pioneering Research Laboratory, E. I. du Pont de Nemours & Co. Inc., Experimental Station, Wilmington, Del.

Session Chairman: H. F. Mark
Institute of Polymer Research
Polytechnic Institute of Brooklyn, Brooklyn, N. Y.

2:00 P.M.-
"Some Light-Scattering Studies of the Texture of Crystalline Polymers" - R. S. Stein, University of Massachusetts, Amherst, Mass.

"Nuclear Resonance Studies of Motion and Configuration in Glassy Polymers" - W. P. Slichter, Bell Telephone Research Laboratories, Inc., Murray Hill, N. J.

Concluding Remarks: H. F. Mark, Institute of Polymer Research, Polytechnic Institute of Brooklyn, Brooklyn, N. Y.

Karl A. Folkers
Chairman

Frederick R. Eirich
Secretary

NEW MEMBERS

Elected May 15, 1959

SUSTAINING MEMBERSHIP

Schneider, Howard, M.D., Medicine. Medical Staff, Yonkers General Hospital, Yonkers, N. Y.

ACTIVE MEMBERSHIP

Barlow, William H., M.D., Medicine. Senior Attending, Staten Island Hospital, Staten Island, N. Y.

Bavin, Eric Morrison, B.Sc., Medicinal Chemistry. Director, Smith & Nephew Research Ltd., Ware, Herts, England.

Beaulnes, Aurèle, M.D., Pharmacology. Head, Pharmacology Department, University of Montreal, Montreal, Canada.

Brendze, Robert Baker, M.D., Orthopedic Surgery. Assistant in Orthopedic Surgery, Harvard Medical School, Boston, Mass.

Brown, S. George, M.D., Pharmacotherapy. Neuropsychiatrist, Concord, N. H.

Budnitz, Edward, M.D., Cardiovascular Diseases. Cardiologist, Worchester City Hospital, Worchester, Mass.

Cameron, Arthur E., M.D., Natural History. Newark, N. J.

Chez, Ronald A., M.D., Obstetrics, Gynecology. Assistant Resident, U.C.L.A. Medical Center, Los Angeles, Calif.

Coen, William B., M.D., Medicine. Private Practice, Springfield, Mass.

Cook, Avery Levem, M.D., Pathology. Instructor in Pathology, Louisiana State University Medical School, New Orleans, La.

Cordero-Judice, Sally Maria, Ph.D., Physiology. Instructor-Biology Department, Marymount College, New York, N. Y.

Creamer, Alan A., V.M.D., Veterinary Medicine. Manager, Veterinary Department Merck, Sharp & Dohme, Inc., West Point, Pa.

De Mello, Walmor Carlos, M.D., Electrophysiology. Associate Researcher, State University of New York, Brooklyn, N. Y.

DesRuisseaux, Pierre, B.A., Neuroanatomy. Student, New York, N. Y.

Diamond, Monroe Thomas, M.D., Internal Medicine. Instructor in Medicine, Cornell University Medical College, New York, N. Y.

Dill, John Nelson, M.D., Internal Medicine. Private Practice, Yonkers, N. Y.

Douglas, Albert H., M.D., Cardiovascular Diseases. Attending Physician, Jewish Hospital, Brooklyn, N. Y.

Dresel, Irmgard, M.D., Pathology. Private Practice of Medicine, Far Hills, N. J.

Durfee, Herbert Ashley, Jr., M.D., Obstetrics, Gynecology, Instructor, University of Vermont College of Medicine, Burlington, Vt.

Edwards, Charles H., M.D., Medicine, Surgery. Private Practice of Surgery, Glen Ridge, N. J.

Eskreis, Naftali, M.D., Medicine. Private Practice of Psychiatry, Forest Hills, N. Y.

Esselstyn, Caldwell B., M.D., Medicine. Executive Director, Rip Van Winkle Clinic, Hudson, N. Y.

Goldberg, Sigmund, M.D., Industrial Medicine, Surgery. Medical Director, Pratt Industrial Clinic, Baltimore, Md.

Gottlieb, Harry, M.D., Diabetes. Assistant Chief, Philadelphia General Hospital, Philadelphia, Pa.

Greif, Martin, B.S., Pharmaceutical. Group Lead, Lederle Laboratories, Pearl River, N. Y.

Guarneri, Victor, M.D., Medicine. Private Practice, Bayside, N. Y.

Heath, James Edward, M.A., Zoology. Interdisciplinary Trainee, University of California, Los Angeles, Calif.

Herrick, Elbert Charles, Ph.D., Organic Chemistry, Chemical Engineering. Supervisor of Chemical Research, Climax Molybdenum Company, Detroit, Mich.

Hoyt, Robert S., M.D., Virology, Immunology. Clinical Instructor in Pathology, Stanford University School of Medicine, Stanford, Calif.

Hudson, Granville W., M.D., Biological Effects of Ionizing Radiation. Associate Professor of Radiology, Vanderbilt University School of Medicine, Nashville, Tenn.

Issekutz, Bela, M.D., Physiology of Metabolism. Research Scientist, Landenau Hospital, Philadelphia, Pa.

Jacobs, Frederick M., M.D., Genitourinary Surgery. Private Practice of Urology, Roanoke, Va.

Jeans, Robert F., M.D., Behavioral Science. Psychiatrist, Chicago Medical School, Chicago, Ill.

Jonsson, Sigurdur, Ph.D., Medicinal Chemistry. Associate Professor of Pharmaceutical Chemistry, University of North Carolina, Chapel Hill, N. C.

Kaplan, Lewis Harvey, M.D., Internal Medicine. Chief Resident in Medicine, Coney Island Hospital, Brooklyn, N. Y.

Kelly, Francis Edward, M.D., Anesthesiology. Adjunct Attending in Anesthesiology, Paterson General Hospital, Paterson, N. J.

Kibler, Raymond S., M.D., Hematology. Assistant Hematologist, Buffalo General Hospital, Buffalo, N. Y.

Kiggins, E. M., Ph.D., Microbiology. Senior Research Microbiologist, Abbott Laboratories, North Chicago, Ill.

Kirschbaum, Edward Harry, M.D., General Surgery. Attending Surgery, Waterbury Hospital, Waterbury, Conn.

Kokernot, Robert H., M.D., Virus Diseases. Staff Member, Rockefeller Foundation, Johannesburg, South Africa.

Kruchek, Thomas Francis, M.D., Psychiatry. Private Practice in Psychiatry, Downers Grove, Ill.

Kuhlmann, Raymond F., M.D., Orthopedic Surgery. Assistant Professor, Burlington, Vt.

Kusz, Clarence V., M.D., Peripheral Vascular Disease. Private Practice, Minneapolis, Minn.

Long, Joseph P., M.D., Gynecology. Assistant Professor, Jefferson Medical College, Philadelphia, Pa.

McCallum, Revell, M.A., Epilepsy, Public Health. Vice-President, United Epilepsy Association, New York, N. Y.

Merritt, Edward G., M.D., Medicine. Private Practice, Dolores, Colo.

Miller, Harry H., M.D., Organ Perfusion. Assistant Professor, Tufts University School of Medicine, Boston, Mass.

Mills, George Thomas, Ph.D., Biochemistry. Associate Professor of Medicine, State University of New York, Brooklyn, N. Y.

Mindlin, Rowland L., M.D., Medicine. Private Practice, White Plains, N. Y.

Misek, Bernard, Ph.D., Biological & Medical Sciences. Pharmaceutical Chemist, Nopco Chemical Company, Harrison, N. J.

Nelson, Waldo E., M.D., Pediatrics. Professor of Pediatrics, Temple University School of Medicine, Philadelphia, Pa.

Ogasawara, Kazuo, M.D., Bacteriology. Professor of Bacteriology, Nagoya University School of Medicine, Nagoya, Japan.

O'Neill, Christopher S., M.D., Cancer. Semi-retired, Chicago, Ill.

Rachelson, Morton H., M.D., Pediatrics. Assistant in Pediatrics, Columbia University, New York, N. Y.

Rafferty, Keen A. Jr., Ph.D., Growth, Embryology. Assistant Professor, Johns Hopkins University, Baltimore, Md.

Richardson, Lionel L., M.D., Mycology. Assistant Medical Director, United Mutual Life, New York, N. Y.

Rutledge, Mary Louise, M.D., Pediatrics. Private Practice, Charlotte, N. C.

Sacerdote, Paul, Ph.D., Internal Medicine. Adjunct Physician, Montefiore Hospital, New York, N. Y.

Sanchez-Martin, J. A., M.D., Endocrinology. Research Fellow, Tufts University, Boston, Mass.

Scherbel, Arthur Lawrence, M.D., Internal Medicine. Head, Department of Rheumatic Disease, Cleveland Clinic Foundation, Cleveland, Ohio.

Schwartz, Samuel H., M.D., Internal Medicine. Senior Attending Physician, Muhlenberg Hospital, Plainfield, N. J.

Shaprio, Raymond N., M.D., Medicine. Clinical Assistant Professor, State University of New York, Brooklyn, N. Y.

Shen, Shu Chu, M.D., Hematology. Assistant Research Professor, Tufts University School of Medicine, Boston, Mass.

Sieber, William K., M.D., Clinical Surgery. Surgeon, Pittsburgh, Pa.

Simms, Leon M., M.D., Neurology, Psychiatry. Private Practice, Brooklyn, N. Y.

Sloan, Robert F., B.A., Skin Homotransplantation. Research Assistant, U.C.L.A. Medical Center, Los Angeles, Calif.

Southerland, William Inzer, M.D., Abdominal Surgery. Vice-President, The Medical & Surgical Clinic, Sherman, Texas.

Speir, Robert Wade, M.D., Virology. Research Fellow in Clinical Virology, Sloan-Kettering Institute for Cancer Research, New York, N. Y.

Steinman, Erwin, M.D., Gastroenterology. Lecturer, Department of Physiology, George Washington University Medical School, Washington, D. C.

Watne, Alvin L., M.D., Cancer Surgery. Associate Cancer Research Surgeon, Roswell Park Memorial Institute, Buffalo, N. Y.

Wirth, Lothar, M.D., Medicine. Private Practice, Rensselaer, N. Y.

STUDENT MEMBERSHIP

Clifford, Margaret A., M.S., Micropaleontology. College Science Assistant, Hunter College, New York, N. Y.

Freudenthal, Anita Regina, M.A., Protozoology. Graduate Student, New York University, New York, N. Y.